Paper No. 45

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

MICHAEL J. ADANG and JOHN D. KEMP

(U.S. Application 07/713,624, filed June 10, 1991),

v.

DAVID A. FISCHHOFF and STEPHEN G. ROGERS

(U.S. Application 07/813,250, filed December 23, 1991).

Interference 103,324

FINAL DECISION

Before: SCHAFER, GRON, and LEE, Administrative Patent Judges.

GRON, Administrative Patent Judge.

Summary of Decision

Entering the priority stage of this interference, Adang was accorded benefit of the October 21, 1988, filing date of U.S. Application 07/260,574 (Adang '88). Fischhoff was accorded

benefit of the November 20, 1986, filing date of U.S. Application 06/932,818 (Fischhoff '86).

The evidence shows that Adang reduced a tomato plant meeting all the limitations of Count 1 to practice by October 29, 1986. Adang's evidence demonstrates that, no later than October 29, 1986, Adang had tomato plants including a full length Bacillus thuringiensis (Bt) crystal protein gene (Bt toxin coding region of ~3.8 kb), and that those plants expressed Bt crystal protein in amounts insecticidal to Lepidopteran insects. Subsequent testing of the plants demonstrated that the Bt crystal protein expressed included Bt crystal protein having a molecular weight of about 130 kD. This demonstrated that Adang's tomato plants were capable of encoding Bt crystal protein of about 130 kD.

Fischhoff's evidence establishes that Fischhoff had a tomato plant which expressed a Bt crystal protein gene (coding sequence of Bt toxin truncated at the DraI site at position 3479, i.e., <3.5 kb) in amounts insecticidal to Lepidopteran insects on October 28, 1986. However, because the evidence of record shows that many Bt crystal proteins ranging in molecular weight from 60-133 kD are toxic to Lepidopteran insects, Fischhoff's evidence does not establish that its tomato plant had been "regenerated from a tomato plant cell transformed to comprise a full length Bacillus thuringiensis crystal protein gene capable of encoding a

Bacillus thuringiensis crystal protein of about 130kD" (Count 1), i.e., Fischhoff failed to prove that the toxic Bt crystal protein its tomato plant produced was not the expression of a truncated Bt crystal protein gene. Therefore, Fischhoff's evidence did not prove that it reduced to practice a tomato plant meeting all the limitations of Count 1 before Adang.

Priority of the invention defined by Count 1 of this interference is awarded to Adang based on its showing that it actually reduced an embodiment meeting all the limitations of Count 1 to practice on October 29, 1986, a date prior to the November 20, 1986, filing date of Fischhoff '86.

Background

September 26, 1983 - Michael J. Adang and John D. Kemp (hereafter Adang) filed U.S. Application 06/535,354 (Adang '83).

April 4, 1986 - Adang filed U.S. Application 06/848,733 (Adang '86) as a continuation-in-part of Adang '83.

November 20, 1986 - David A. Fischhoff and Stephen G. Rogers (hereafter Fischhoff) filed U.S. Application 06/932,818 (Fischhoff '86).

October 21, 1988 - Adang filed U.S. Application 07/260,574 (Adang '88) as a continuation-in-part of Adang '86.

June 10, 1991 - Adang filed involved U.S. Application 07/713,624 (Adang '91) as a continuation of Adang '88.

December 23, 1991 - Fischhoff filed involved U.S. Application 07/813,250 (Fischhoff '91) as a continuation of Fischhoff '86.

February 28, 1994 - Interference 103,324 was declared between Claims 11, 12, 15, 16, 20, 21, 24, 25, 38, 39, 42, and 43 of Fischhoff '91 and Claims 16, 17, 22, 25-27, 29-32, 34, 40, 43, 46-50, and 57 of Adang '91 (Paper No. 2) which were designated as corresponding to the following Count 1:

Count 1

> A tomato plant which has been regenerated from a tomato plant cell transformed to comprise a full length Bacillus thuringiensis crystal protein gene capable of encoding a Bacillus thuringiensis crystal protein of about 130 kD under control of a promoter such that said gene is expressible in said plant in amounts insecticidal to Lepidopteran insects.

Adang initially was accorded benefit of the October 21, 1988, filing date of Adang '88 and the April 4, 1986, filing date of Adang '86 for purposes of establishing priority of the invention defined by Count 1. Fischhoff initially was accorded benefit of the November 20, 1986, filing date of Fischhoff '86.

September 29, 2000 - The Board of Patent Appeals and Interferences (Board) entered a Final Decision (Paper No. 233):

(a) holding that (Paper No. 233, p. 19):

"tomato plants encompassed by Count 1 (1) must have been regenerated from a tomato plant cell transformed by a full length Bt crystal protein gene which encodes Bt crystal protein of about 130 kD under control of a promoter which directs expression of said structural gene in said tomato plant cell, and (2) must produce amounts of Bt crystal protein protoxin of about 130 kD which destroy or control Lepidopteran insects in any way[;]"

(b) granting Fischhoff's Preliminary Motion 1 (Paper No. 35) under 37 CFR § 1.633(c)(3) to designate Claims 15, 24, 42, and 44 of Adang '91 as corresponding to Count 1;

(c) denying Fischhoff's Preliminary Motion 2 (Paper No. 36) under 37 CFR § 1.633(a) for judgment that Claims 16, 22, 25-27, 29-32, 40, 43, 46, and 47-50 of Adang '91 are unpatentable under 35 U.S.C. § 112, first paragraph;

(d) denying Fischhoff's Preliminary Motion 3 (Paper No. 37) under 37 CFR § 1.633(a) for judgment that Claims 15, 24, 42, and 44 of Adang '91 are unpatentable under 35 U.S.C. § 112, first paragraph;

(e) granting Fischhoff's Preliminary Motion 11 (Paper No. 23) under 37 CFR § 1.633(g) attacking the benefit of the April 4, 1986, filing date of Adang '86 originally accorded Adang for Count 1;

(f) denying Fischhoff's Preliminary Motion 13 (Paper No. 25) under 37 CFR § 1.633(c)(2) to add new Claims 44, 45, and 46 to Fischhoff '91 and designate the new claims as corresponding to Count 1;

(g) dismissing Fischhoff's Preliminary Motion 14 (Paper No. 27) under 37 CFR § 1.633(f)(2) to accord new Claims 44, 45, and 46 benefit of the November 20, 1986, filing date of Fischhoff '86;

> (h) denying Adang's Preliminary Motion 1 (Paper No. 15) for benefit for purposes of priority of the subject matter defined by Count 1 of the September 26, 1983, filing date of Adang '83; and inter alia,
>
> (i) denying Adang's Preliminary Motion 2 (Paper No. 16) under 37 CFR § 1.633(a) for judgment that Claims 11-12, 15-16, 20-21, 24-25, 38-39, and 42-43 of Fischhoff '91 are unpatentable under 35 U.S.C. § 102 and/or § 103.

Considering the evidence of record relative to its interpretation of the subject matter defined by Count 1 and its decisions on motions, the Board concluded that Adang had not established actual or constructive reduction to practice of an embodiment of Count 1 prior to the November 20, 1986, filing date of Fischhoff '86. Since Adang did not present a case for prior conception of the invention of Count 1 coupled with diligence to filing Adang '88, the Board "ORDERED that judgment on priority as to Count 1 . . . is awarded against party . . . Adang" (Paper No. 223, p. 109).

April 10, 2002 - The Court of Appeals for the Federal Circuit concluded in Adang v. Fischhoff, 286 F.3d 1346, 62 USPQ2d 1504 (Fed. Cir. 2002), at 1359-60, 62 USPQ2d at 1514:

> The Board erred in construing Count 1 by requiring the insecticidal effect of the transformed tomato plants of the count to be attributed solely to sufficient amounts of the 130 kD Bt crystal protein protoxin. It is sufficient that the crystal protein that produces the insecticidal effects in the plants

> of the count be the result of the expression of the full length protein gene; the size of the resulting protein is immaterial. Accordingly, we reverse the Board's count construction in this regard. The factual findings of the Board on the question of whether Adang '86 would have enabled persons skilled in the art to make and use the transformed tomato plants of the count as of the filing date of the application are supported by substantial evidence. Even under the modified count construction, these findings indicate that undue experimentation would have been required to produce the tomato plants of the count on the basis of the disclosure of Adang '86. We thus affirm the Board's legal conclusion that Adang '86 was not an enabling disclosure and that Adang was not entitled to rely on the April 4, 1986 filing date of that application to establish priority. Finally, we are unable to determine on the basis of the record on appeal whether Adang established entitlement to a priority date before November 20, 1986. Accordingly, we remand the case to the Board for further consideration of this issue in light of the modified count construction.

Preliminary Remarks

In the "Proposal and Order" entered April 25, 2002 (Paper No. 236), an Administrative Patent Judge (APJ) advised the parties of the Board's proposal to finally determine priority of the invention of the interference count based on the existing record and invited the parties to comment (Paper No. 236, p. 2). In the "Record of Telephone Conference and Order" entered June 13, 2002 (Paper No. 245), the APJ reported (Paper No. 245, p. 2):

> The parties previously had been invited to comment on the APJ's proposal to finally determine priority of the invention of the interference count, as interpreted

> by the Federal Circuit, based on the existing record (Paper No. 236). Fischhoff had filed a summary reply assenting to the proposal (Paper No. 240). Adang had filed a comprehensive response (~30 pages) not only assenting to the proposal but also discussing the merits of the proposed determination of priority of the invention of the count based on the existing record and requesting permission to file a Rule 633(g) motion attacking the benefit of the filing date of Fischhoff's 1986 application originally accorded Party Fischhoff (Paper No. 239).

The APJ (1) granted Fischhoff permission to comment on Adang's response (Paper No. 239), (2) denied Adang's request to belatedly file a preliminary motion under Rule 633(g), and (3) granted Adang permission to respond to Fischhoff's additional commentary and preserve its right to file the proposed motion under Rule 633(g)(Paper No. 245, pp. 3-4). Neither party requested filing additional evidence. Accordingly, priority of the invention of the interference count, as interpreted by the Federal Circuit, hereafter shall be finally determined based on the existing record and additional comments by the parties.

Discussion

In its interpretation of the scope of Count 1 in Adang, the Federal Circuit held, 286 F.3d at 1355, 62 USPQ2d at 1510:

> [T]he tomato plants encompassed by Count 1: (1) must have been regenerated from a tomato plant cell transformed by a full length Bt crystal protein gene which encodes Bt crystal protein of about 130 kD under control of a promoter which directs expression of said structural gene in said tomato plant cell, and (2) must produce amounts

of a Bt crystal protein of any size which destroy or control Lepidopteran insects in any way.

With regard to priority of the invention defined by Count 1, the court said, Adang, 286 F.3d at 1358-1359, 62 USPQ2d at 1513-14:

> Since Adang '86 is not enabling. Adang's case for priority of invention of Count 1 rests on an ability to show that Adang had either actually reduced to practice an embodiment of the count prior to the November 20, 1986, filing date of Fischhoff '86, or had conceived of an embodiment of the count prior to that date and was diligent in reducing the embodiment to practice. As the presumptive junior party, Adang had the burden to show priority of invention by a preponderance of the evidence. . . . The Factual findings of the Board are insufficient to determine whether Adang has sustained his burden of proof on this issue.
>
> The Board's factual findings focused on Adang's failure to show that the 130 kD crystal protein protoxin was expressed in the transformed plants. Thus, for example, the Board found that the bioassays in Adang '88 "test for toxic Bt crystal protein produced by the plants, not how much 130 kD crystal protein the plants produced." Board opinion, slip op. at 99. As noted above, however, under the correct construction of Count 1 it is immaterial which form of the crystal protein is produced, so long as it is clear that the protein results from the expression of the full length Bt crystal protein gene, which is "capable of encoding a ... crystal protein of about 130 kD." (emphasis added). In other words, the toxic protein must not be the result of the expression of a truncated gene, which would not be capable of encoding the full 130 kD protoxin.
>
> The Board's factual findings on this point are somewhat confused. On the one hand, the Board seems to settle the identity of the gene incorporated in the

plasmid used to transform the tomato plants: "In our view, the evidence shows that Adang transformed tomato cells using a pH450 vector which includes a full length Bt crystal protein gene encoding Bt crystal protein of about 130 kD." Board opinion, slip op. at 104. On the other hand, the Board expresses some doubt as to whether the tomato plants incorporated the entirety of this gene. "[T]he tomato plant cells may have been transformed by the full length Bt crystal protein gene which encodes Bt crystal protein of about 130 kD and/or a truncated fragment thereof." Id. at 104-05 (emphasis added). The Western blot evidence that the Board cites from Adang '88, furthermore, is unclear; although the specification states that "[b]oth protoxin and toxin was observed in leaf tissues," Western blot results for two transformants showed "smearing at about 110 kD." In other words, the assay failed to show any of the 130 kD protoxin in plants regenerated from those two transformants. Although Adang argues that all limitations of the count were met at the latest by June 12, 1986, on which date bioassays were allegedly conducted which showed that tomato plants transformed with the full length Bt crystal protein gene were toxic to Lepidopteran insects, we are unable to confirm this from the record on appeal.

1. Adang's case for priority of invention

A. Precedent

A junior party seeking a determination of priority must demonstrate by a preponderance of the evidence reduction to practice before the senior party's priority date or prior conception coupled with reasonable diligence in reducing the invention to practice from a time just before the senior party entered the field to the junior party's own reduction to practice. 35 U.S.C. § 102(g) (1994); Mahurkar v. C.R. Bard, Inc., 79 F,3d 1572, 1577, 38 USPQ2d 1288, 1290 (Fed. Cir. 1996).

Griffin v. Bertina, 285 F.3d 1029, 1032, 62 USPQ2d 1431, 1433 (Fed. Cir. 2002). As in Estee Lauder Inc. v. L'Oreal S.A.,

129 F.3d 588, 44 USPQ2d 1610 (Fed. Cir. 1997), whether junior party Adang was both first to conceive the invention and reasonably diligent in reducing it to practice is not before us. Adang has not presented a case for priority based on conception plus reasonable diligence to actual or constructive reduction to practice. The question before us is whether Adang has shown by a preponderance of the evidence that it actually reduced an embodiment encompassed by the interference count to practice prior to the November 20, 1986, filing date of Fischhoff '86.

> To prove actual reduction to practice, an inventor must establish that he "'actually prepared the composition and knew it would work.'" Hahn v. Wong, 892 F.2d 1028, 1032, 13 USPQ2d 1313, 1317 (Fed. Cir. 1989)(quoting Mikus v. Wachtel [II], 542 F.2d 1157, 1159, 191 USPQ 571, 573 (CCPA 1976).

Estee Lauder Inc. v. L'Oreal S.A., 129 F.3d at 592, 44 USPQ2d at 1613.

> See Newkirk v. Lulejian, 825 F.2d 1581, 1582, 3 USPQ2d 1793, 1794 (Fed. Cir. 1987)("[E]very limitation of the interference count must exist in the embodiment and be shown to have performed as intended.") . . . .

Schendel v. Curtis, 83 F.3d 1399, 1402, 38 USPQ2d 1743, 1746 (Fed. Cir. 1996).

The inventor may establish that it actually prepared an invention encompassed by the count by later evidence only when the evidence of record as a whole establishes that the inventor

contemporaneously recognized or appreciated that it had actually prepared an invention encompassed by the count. There can be no conception or reduction to practice of an invention where there has been no recognition or appreciation of the invention. Silvestri v. Grant, 496 F.2d 593, 597, 181 USPQ 706, 708 (CCPA 1974), cert. denied, 420 U.S. 928 (1975). An inventor may rely on later developed evidence to confirm that an invention that it contemporaneously recognized and appreciated had been actually prepared. Silvestri v. Grant, 496 F.2d at 599-601, 181 USPQ at 709-711. See Mycogen Plant Science, Inc. v. Monsanto Co., 243 F.3d 1316, 1334, 58 USPQ2d 1030, 1046 (Fed. Cir. 2001) ("conception and reduction to practice cannot be established nunc pro tunc. There must be contemporaneous recognition and appreciation of the invention represented by the counts." Breen v. Henshaw, 472 F.2d 1398, 1401, 176 USPQ 519, 521 (CCPA 1973)(emphasis added)). Hitzeman v. Rutter, 243 F.3d 1345, 1358, 58 USPQ2d 1161, 1171 (Fed. Cir. 2001), teaches:

> Nunc pro tunc conception involves the situation where an inventor actually possessed a claimed . . . [invention] at the time of his alleged conception, but failed to recognize . . . [its] inventive features at the time. . . . [A]n inventor who failed to appreciate the claimed inventive features . . . at the time of alleged conception cannot use his later recognition of those features to retroactively cure his imperfect conception.

Cooper v. Goldfarb, 154 F.3d 1321, 47 USPQ2d 1896 (Fed. Cir. 1998), clarifies at 1331, 47 USPQ2d at 1904:

> The rule that conception and reduction to practice cannot be established nunc pro tunc simply requires that in order for an experiment to constitute an actual reduction to practice, there must have been contemporaneous appreciation of the invention at issue by the inventor. . . . Subsequent testing or later recognition may not be used to show that a party had contemporaneous appreciation of the invention. However, evidence of subsequent testing may be admitted for the purpose of showing that an embodiment was produced and that it met the limitations of the count. See Silvestri v. Grant, 496 F.2d 593, 598, 181 USPQ 706, 709 (CCPA 1974).

Ultimately, "a reduction to practice does not occur until the inventor has determined that the invention will work for its intended purpose." Estee Lauder Inc. v. L'Oreal S.A., 129 F.3d at 592, 44 USPQ2d at 1614. "[W]hen testing is necessary to establish utility, there must be recognition and appreciation that the tests were successful for reduction to practice to occur." Estee Lauder Inc. v. L'Oreal S.A., 129 F.3d at 593, 44 USPQ2d at 1615.

Accordingly, Adang may rely on the results of its April 1997 Western blot analysis of the expression products of regenerated tomato plant cells transformed with Bt crystal protein gene which were insecticidal to Lepidopteran insects to confirm that Adang had actually prepared an embodiment meeting all the limitations

of the interference count. Our reviewing court instructed in Adang, 286 F.3d at 1355, 62 USPQ2d at 1510, that to establish priority based on an alleged actual reduction to practice, the preponderance of the evidence must not only show that Adang prepared a tomato plant which was "regenerated from a tomato plant cell transformed to comprise a full length Bt crystal protein gene which encodes Bt crystal protein of 130 kD under control of a promoter which directs expression of said structural gene in said tomato plant cell," it must show that the tomato plants Adang prepared "produce amounts of a Bt crystal protein of any size which destroy or control Lepidopteran insects in any way," before the November 20, 1986, filing date of Fischhoff '86. Only after Adang establishes that it actually reduced an embodiment encompassed by the interference count to practice before the November 20, 1986, filing date of Fischhoff '86, need we consider whether Fischhoff reduced an embodiment to practice before Adang.

B. Evidence and findings

"As the presumptive junior party, Adang had the burden to show priority of invention by a preponderance of the evidence." Adang, 286 F.3d at 1359, 62 USPQ2d at 1513. "Although Adang argues that all limitations of the count were met at the latest

by June 12, 1986, on which date bioassays were allegedly conducted which showed that tomato plants transformed with the full length Bt crystal protein gene were toxic to Lepidopteran insects, we are unable to confirm this from the record on appeal." Adang, 286 F.3d at 1359, 62 USPQ2d at 1514.

We proceed to determine whether the preponderance of the evidence of record establishes that Adang actually reduced an invention encompassed by the interference count to practice any time prior to the November 20, 1986, filing date of Fischhoff '86. To that end, we examine the factual basis for the statements made at pages 17-18 of Adang's Brief at Final Hearing (AB 17-18)(Paper No. 223) in support of its argument that "Adang Actually Reduced To Practice The Subject Matter Of The Count Prior To Fischhoff" (AB 17):

> On February 2 and 3, 1984, Dr. [Andrew] Binns [of the University of Pennsylvania] met in Madison, Wisconsin, with Dr. [John D.] Kemp, Dr. [Michael J.] Adang and Dr. Donald Merlo. (See "Sponsored Research Report," AX-021, last page section I.) During the meeting, these individuals arranged a collaborative project wherein personnel in Dr. Binns' laboratory would use vectors containing the B.t. crystal protein gene, developed at Agrigenetics, to transform tomato plants and perform assays to evaluate the expression of the B.t. crystal protein gene in tomato plants. (AD-0528)[(AB 17)].

This statement (AB 17) is taken substantially verbatim from paragraph 6 (AD 0528) of the Declaration by Andrew N. Binns,

dated June 30, 1997 (AD 0527). Dr. Binns declared that he received A. tumefaciens strain 014-RS_2 from an Agrigenetics employee supervised by Dr. Kemp on February 3, 1984. The plan was to employ the 014-RS_2 vector (a) "to develop a 'root cloning' technique," and (b) "as a plant expression vector in the transformation and regeneration of tomato plants containing an insecticidal B.t. crystal protein gene" (AD 0528-0529). Dr. Binns declared (AD 0529):

> At the time, I understood that Kemp, Adang, Merlo and Carolyn Stock ("Stock") were developing a plant expression cassette that contained the native full-length B.t. crystal protein gene which, once completed, would be inserted into the 014-RS_2 vector.

Dr. Binns declared (AD-0530):

> 9. In early September 1984, I received a letter and enclosed culture of A. tumefaciens LBA4404 from Merlo. (Adang Exhibit 119). LBA4404 harbored a micro Ti (pAN-6) containing an untested plant-selectable kanamycin resistance gene. At the time, it was my understanding that Kemp, Adang and Merlo intended to develop a micro Ti construct for the purpose of developing transgenic plants expressing an insecticidal B.t. crystal protein. Merlo provided the material to me in September 1984 so that I could begin testing the pAN-6 construct in tomato, in anticipation of later receiving the micro Ti plasmid containing the B.t. crystal protein gene.
>
> 10. On or about November 21, 1984, I received a letter and enclosed cultures RS_2-014 and R3-11 from Stock. (Adang Exhibit 120). Stock explained that $RS_2$014 was a rooty-shooty mutant derived from the strain 15955 (octopine-type Agro) and R3-11 was the mannopine/

B.t. construct in T-right of RS_2014. . . . Stock noted that R3-11 in plants still produced mannopine and/or argopine and, in Agrobacterium, it had been shown to produce truncated forms of a B.t. crystal protein.[1] I began transforming tomato tissue with RS_2-014 and R3-11 shortly thereafter. Sara Tatchell Patterson ("Patterson") and I routinely reported the results of our work with this material to Adang and others working on the B.t. project at Agrigenetics.

Adang argues (AB 17):

> On March 28, 1985, Dr. Merlo provided to Dr. Binns samples of the pH450 construct, which Dr. Binns understood to contain the full length B.t. crystal protein gene under the control of the mannopine synthase promoter (the control of a plant expressible promoter)[(AD-0531, para. 11); (AD-1379, para. 8-9); (AD-1382, para. 13a); and (AD-1444)].

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[1] The material portions of the letter read (AX 120):

November 21, 1984

Dear Andy:

Enclosed are the Agro strains you requested . . . . RS_2014 is Paula Chee's rooty-shooty mutant derived from the strain 15955 (octopine-type Agro). R3-11 is the mannopine - BT construct in T-right of RS_2014. This construction on plants will still produce mannopine and/or agropine and in Agrobacterium, it has been shown to produce truncated forms of B.T. crystal protein.

Please keep in mind that the data we have on expression in plants is still suggestive. We are hoping to do polyA RNA isolations from plant tissues next week and will notify you of those results as soon as they are available.

Sincerely,

Carolyn Stock

Cited in support of Adang's argument are

(1) paragraph 11 of Dr. Binns' declaration (AD 0531) (emphasis added):

> 11. On March 28, 1985, I met with Merlo in my laboratory at the University of Pennsylvania. Merlo gave me samples of LBA4404(pAN/kan-1) and LBA4404(pH450) constructs. At that time, I understood that the pH450 construct contained the mannopine promoter and full-length B.t. crystal protein gene. I began transforming tomato tissue with (pAN/kan-1) and pH450 shortly thereafter. Patterson and I routinely reported the results of our work with this material to Adang and others working on the B.t. project at Agrigenetics.

(2) paragraphs 8 and 9 of the Declaration Under 37 C.F.R. §1.672(b) by Donald J. Merlo, dated June 25, 1997 (AD 1379):

> 8. The purpose of developing these constructs was to provide binary vectors with the following main features:
>
> i) could replicate in both Escherichia coli and Agrobacterium tumefaciens cells;
>
> ii) possessed an antibiotic resistance gene that functioned in both types of bacterial cells;
>
> iii) could be mated by tri-parental conjugation from E. coli to Agrobacterium;
>
> iv) contained left and right T-DNA borders derived from the parent plasmid pTi-15955;
>
> v) contained a plant-expressible gene that functioned as a selectable marker for transformed plant cells following stable integration into a plant genome;
>
> vi) conferred no abnormal phenotypes to the transformed plant cells that harbored it;
>
> vii) contained a unique restriction site situated between the selectable marker gene and the right T-DNA border; and
>
> viii) had a total size of less than about 20 kilobase pairs (kbp).

9. One of the main purposes of developing these plasmids was for using them as vectors to introduce chimeric Bacillus thuringiensis (Bt) genes encoding toxic proteins into plant cells. The basic vectors were pH400 and pH575. Into these basic vectors were cloned Bt genes under the control of various plant promoters, thereby generating plasmids including pH450, pH577, pH578 and pH582.

(3) paragraph 13a of Dr. Merlo's declaration (AD 1382):

13a. In the 1/22/85 meeting, it was decided that we would clone a BglII fragment containing a Bt toxin gene under the control of the mannopine synthase promoter into the unique BglII site of pH400. I indicated on (DJM 278/6) that the source of the "man/Bt" gene would be plasmid "pCJ161", prepared by Dennis Sutton and recorded in (DS 230/38). The size of the Bt (HD73) coding region in this construct is approximately 3.78 kbp and, when combined with the mannopine synthase promoter and associated 3' untranslated sequences, the total fragment had a size of approximately 5.96 kbp.

(4) Part II - Detailed Chronology, paragraph 1, of the Declaration Under 37 C.F.R. §1.672(b) by Sara E. (Tatchell) Patterson, dated June 30, 1997 (AD 1444-1445):

1. Transformation of Tomato Plant Tissue With Full-length B.t. Insecticidal Gene

In November, 1984, (AB 55/68) experiments were initiated which provide a successful hypocotyl transformation using R3-11, one of the earliest vectors developed containing the native, full-length B.t. gene. Since there was no resistance for kanamycin or other antibiotics, selection for transgenics was strictly on the basis of opine production. Opine positive tissues were identified on February 1, 1985, subcloned and reassayed for opine production.

On March 5, 1985, these tissues were expanded for cloning and ultimately regenerated into plants. Regenerated plants from 9C-G2 were assayed for biological activity (ST 1188/1-3)[2] and determined

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[2] Pages 1 and 2B of Sara Tatchell's Laboratory Notebook No. 1188 appear to have been signed by S. Tatchell on October 26, 1986, and signed on November 5, 1986, as disclosed to and understood by, E. Dale. Pages 3A-3C of the same laboratory notebook appear to have been signed by S. Tatchell on October 29, 1986, and signed on November 5, 1986, as disclosed to and understood by, E. Dale (AX 81, Book No. 1188, pp. 1-3). Page 1 of Sara Tatchell's Laboratory Notebook 1188 has the following handwritten entries (AX 81, Book No. 1188, p. 1)(* = illegible):

TITLE Bioassays of 'UC82' Lycopersicum esculentum with Manduca sexta tobacco hornworm for Bt protein PROJECT NO. 9A1 BOOK NO. 1188

PURPOSE: To determine if putative Bt protein containing R_0 and R_1 plants transformed with pH450 (exp. 55A71) are resistant or sensitive to Manduca sexta (Tobacco hornworm were ordered from Carolina Biological Supply).

PROTOCOL: 1) R_0 and R_1 plants have been isolated from normal greenhouse facilities to avoid greenhouse spraying.

2) Eggs are ordered from Carolina Biological Supply and placed on plants and leaf discs. (Both whole plants & leaf discs on filter paper assayed)
Each plant assayed receives 5 hornworms/plant
plates receive $\geq$5 hornworms/plate
#s recorded

10/23/86

Eggs received

to provide some insecticidal activity, as tobacco hornworms displayed reduced growth and 40% to 80% mortality. See Figure 5, attached. Additional experiments with R3-11 transgenic lines were not pursued due to the number of new transgenics initiated from alternative vectors, pH450, pH577, and pH578.

In April, 1985, we initiated experiments which resulted in the successful transformation of tomato plant tissue with pH450, one of the earliest binary vectors containing the full-length native B.t. gene. This transformation was one of the first attempts of Binns' laboratory to insert pH450 into tomato plant and it was successful. Growth of the transformed plant tissue on the selective antibiotic G418 and opine expression were criteria used to initially identify transgenic tissues.

In this transformation, we determined that varying levels of G418 (0 to 20ug/L) could be used to successfully select for transformants. The plant tissue, transformed with pH450, was tested for octopine production by incubating tissue samples on medium containing arginine. Opine producing tissues from

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10/24/86

Eggs placed with plant material (early morning)
Some eggs beginning to hatch Mid -> late afternoon - evening some worms observed dead
1 on IC82 leaf
1 on 55PH-71-4-***
All plates quantified at this point-# worms/plate

10/25/86

10/26/86 Photographs taken of all plates/observations recorded.
food supply plate #1 and #4 quite depleted

Photograph negatives on Pages 2A and 3C are blank. Pages 2B and 3B present handwritten data. Page 3A presents a bar graph of "Data from 'Bio assay #1'".

the transformed plant samples were identified, subcultured and regenerated into plants.

Adang argues (AB 17-18)(emphasis added):

> In April, 1985, Dr. Binns initiated successful transformations of tomato plant tissue using the pH450 construct. [See Sara (Tatchell) Patterson's declaration (AD-1444).]
>
> On March 11, 1986, Dr. Binns initiated ELISA assays using samples from tomato plants transformed with pH450 and monoclonal antibodies for a B.t. crystal protein toxin (HD73). On March 20, 1986, Dr. Binns determined that the tissue from plants transformed with pH450 were positive for the expression of B.t. crystal protein. (AD-1476)[.]

In support of Adang's latter argument, Sara (Tatchell) Patterson declared (AD 1476-1477)(emphasis added):

> a. ELISA No. 1: Detection of B.t. Protein in Tomato Plants Transformed With pH450
>
> The enzyme-linked immunosorbent assay (ELISA) was used to assay for the expression of B.t. insecticidal crystal protein in tomato plants transformed with pH450. The plant tissue tested in this ELISA was taken from UC82 transgenics originating from the pH450 hypocotyl transformation initiated on February 2, 1985 (AB 55/71).
>
> The ELISA was performed according to a standard protocol described in Part III, subsection 7, of this declaration, and detailed below according to the date the various steps of the protocol were performed.
>
> (1) On March 11, 1986, (ST 985/15) microliter plates were coated with mouse monoclonal anti-B.t. HD73 antisera obtained from Agrigenetics.
>
> (2) On March 12, 1986, (ST 985/15) plant tissue samples from UC82 were prepared for assay, the protein

concentration of each sample was determined, and then each sample was assayed for B.t. protein using the ELISA.

Also on March 12, 1986, (ST 985/15) the identification number for each tissue sample was recorded along with the results of the ELISA. In addition, the measurements from the plate reader and readout were recorded on March 12, 1986 (ST 985/16).

b. ELISA No. 2: Detection of B.t. Protein in Tomato Plants Transformed With pH450

The ELISA was used to assay for the expression of B.t. insecticidal crystal protein in tomato plants transformed with pH450. The results of this ELISA demonstrates the expression of B.t. insecticidal protein in UC82 tomato plants transformed with pH450. The plant tissue tested in this ELISA was taken from UC82 transgenics originating from the pH450 hypocotyl transformation initiated on February 2, 1985 (AB 55/71).

The ELISA was performed according to a standard protocol described in Part III, subsection 7, of this declaration, and detailed below according to the date the various steps of the protocol were performed.

(1) On March 11, 1986, (ST 985/17) microliter plates were coated with mouse monoclonal anti-B.t. HD73 antisera provided by Agrigenetics.

(2) On March 19, 1986, (ST 985/17) plant tissue samples from UC82 transgenics were prepared for assay, the protein concentration of each sample was determined, and then each sample was assayed for B.t. protein using the ELISA.

(3) On March 19 and 20, 1986, (ST 985/17) the results of the protein absorbance readings and ELISA were recorded. In addition, a copy of the print out from the plate reader and a grid indication the plate arrangement was recorded (ST 985/18).

Pages 15 and 16 of Sara Tatchell's Laboratory Notebook No. 985 (AX 81) to which Sara E. Patterson refers appear to have been signed by Sara Patterson Tatchell on July 24, 1986, and signed on August 5, 1986, as disclosed to and understood by A. Binns. Pages 17 and 18 of Sara Tatchell's Laboratory Notebook No. 985 (AX 81) appear to have been signed by Sara Patterson Tatchell on March 22, 1986, and signed on August 5, 1986, as disclosed to and understood by A. Binns. With regard to pages 15 and 16 of Sara Tatchell's Laboratory Notebook No. 985 (AX 81), Sara E. Patterson merely declares (AD 1476-1477) that "each sample [of B.t. transformed tomato leave tissue] was assayed for B.t. protein using the ELISA" and the results are reported at ST 985/15-16.

We find in Sara E. Patterson's declaration (AD 1476) with regard to ST 985/15-16 or in ST 985/15-16 itself, no express statement that data there presented shows that transformed UC82 tissue tested positive for B.t. crystal protein using ELISA "in amounts insecticidal to Lepidopteran insects" (Count 1). Citing data presented on pages 17 to 18 of Sara Tatchell's Laboratory Notebook No. 985 (AX 81), Sara E. Patterson declared (AD-1477):

> On March 20, 1986, (ST 985/17, 18) I concluded from the results of the ELISA that the plant tissue sample designated L-1 (transformed with pH450) was

positive for the expression of B.t. protein. Specifically, the absorbance readings of the protein detected by the ELISA indicated that plant L-1 contained approximately 4.95 ug B.t. protein/7.5 mg total protein/g tissue.

Neither the conclusion "from the results of the ELISA that the plant tissue sample designated L-1 (transformed with pH450) was positive for the expression of B.t. protein" (AD-1477) nor the statement that "the absorbance readings of the protein detected by the ELISA indicated that plant L-1 contained approximately 4.95 ug B.t. protein/7.5 mg total protein/g tissue" (AD-1477) are taken from Laboratory Notebook pages ST985/17 or ST985/18. Between Laboratory Notebook pages ST985/17 or ST985/18, we find what appears to be a two-part insertion without page number or title. The first part of the insertion reads as follows (AX 81; * = unclear):

3/20/86
BT Standards in Tissue

500µg	.546;	.489	.5175	**********				
250	.377	.341	.359					
100	.137	.133	.135					
50	.070	.050	.110					
UC82	.154	.081	.140	.117	.132	.040	.068	\.099
	.024	.154	.173	.020	.137	.080		/
PH450 L-1 #3	.029	.092	.103	.103	.028	.000	.050	
PH450 L1 #4	.0	.108	.134	.173	.172		.147	
#5 PH450 V-1 tis -	.032	.004	.009	.081	.029			
#6 2A76 tis	.063	.025	-.009					
#7 PH450 V-1 tis	-.036	-.020						
4A4#16 A tis	.007	.039	.044	-.025	.051			

Conclusions

L-1 positive

~100µg BT/150µg protein = .66µg/µg protein

= 4.95µg BT/7.5µg protein/g tissue

The second part of the insertion is a plot of the following data:

BSA Standards	OD
10µg	.098
20	.236
40	.608
60	.664
80	.722

Leaf protein (4x)	
50λ	1.606
40λ	1.530
30λ	1.427
20λ	1.133
10λ	.784
5λ	.370
Blank	.009.

At the bottom of the second part of the insertion appear the handwritten entry "protein assay 3/19/86" and the dated signature of "S. Tatchell 7/24/86" (AX 81, Laboratory Notebook ST985, insert between pages 17 and 18 thereof). The insertion is not signed.

Adang's argument continues (AB 18):

> In June 1986 Dr. Binns began sending Dr. Adang seeds from pH450-transformed tomato plants, and tissues from the transformed plants. (AD-0373, para. 130, citing AX-006, Notebook 336, p. 140). More seeds were sent in November 1986 from pH450 tomatoes called ST-4-5 and ST-2-4 (AD-0396, para. 22, citing AX-006, Notebook 405, p56).

In the Declaration of Michael J. Adang, dated July 1, 1997 (AD-0020-04463), Dr. Adang discusses Notebook 336 (AD-0332-0384). Dr. Adang declared (AD-0373):

> 130. Page 140, is entitled "Tomato tissues from Binns." I dated my entry on 6/12/86. Andy would send me capped test tubes containing agar with tomato tissues. In this case he sent also me some leaf and stem tissue wrapped in wet towels. Sara Tatchell had done some experiments with these tissues where she was feeding them to worms. Sara was seeing some insect toxicity and Andy wanted us to also perform bioassays. On this page, I attached the actual labels that I had taken from the tubes. pH450 was a construct containing a mannopine promoter and full length BT construct that they had put into tomato. Andy also sent me some seeds. Page 140 is just a record of my receipt of these tissues. I signed page 140 on 6/18/86. Hoffman signed it on 11/10/86, and Rocheleau signed it on 10/15/86.

Page 140 of Adang's Notebook No. 336 (AX-006), to which Dr. Adang refers, reads (* = illegible):

140 Book No. 336 TITLE Tomato Tissues From Binns

6/12/86
nontransformed (UC82)

L-1 (pH450#58) LK82
M-1 (pH450
/
Rec'd via express leaf
& stem tissue wrapped
in wet towels
/
Placed Thu* on
leaf tissue
/
Put tissues ** 50ml
conical tubes & added

Liq.** then put **
caps loose at -70°C
/
Seeds: 4P-V7R-7-8
4A7 *9 M-1

10
only got 8* packet.

Dr. Adang signed page 140 on June 18, 1986. Hoffman signed it on November 10, 1986, and Rocheleau signed it on October 15, 1986. Adang's Notebook No. 336, p. 140, does not indicate whether Dr. Adang's declarations that "Sara Tatchell had done some experiments with these tissues where she was feeding them to worms" and "Sara was seeing some insect toxicity" (AD-0373) refer to events which occurred prior, contemporary, or subsequent to either June 12, 1986, or the November 20, 1986, filing date of Fischhoff '86.

Adang argued (AB 18)(emphasis added):

> On June 12, 1986, Dr. Adang received tomato tissues from Dr. Binns. These samples included leaf and stem tissues from plants that had been transformed with pH450. Dr. Adang appreciated that Dr. Binns had conducted bioassays on these tissues and observed insect toxicity. (AD-0373, para. 130). Moreover, Dr. Adang appreciated that the full length B.t. gene contained in pH450 encoded a crystal protein toxin of about 130kD. [(AD-0373, para. 129 and 130) and (AD-1479)] These plants constituted an actual reduction to practice of tomato plants within the scope of the Count.

As previously indicated, Adang's Brief cites AD-0373, para. 130, which cites Adang's Notebook 336, p. 140 (AX-006). Adang's Notebook 336, p. 140, does not establish that bioassays had been run on tomato plants for insect toxicity or insect toxicity had been observed any time prior to November 20, 1986. Nor does Adang's Notebook 336, p. 140, itself establish that Dr. Adang had confirmed that the B.t. gene contained in pH450 encoded a crystal protein toxin of about 130kD any time prior to November 20, 1986. Also cited in Adang's declaration, dated July 1, 1997, paragraph 129 thereof (AD-0372) reads (emphasis added):

> 129. Pages 136-139 are not titled. Pages 136-139 are all a related experiment. In this experiment, I prepared a toxin by trypsin activation of HD73 crystals. I ran a column and concentrated the fractions from the column where the protein peak was. I determined the amount of protein toxin that came from this peak and I stored the toxin for later use. The actual gel for my toxin preparation is in my book 405, page 11. On page 138, I listed at the top of the page the toxins that I had stored away at -20°C. I analyzed these toxins to look at the size of the proteins in them to make sure they were still there. At the bottom of the page I attached the SDS-PAGE where the toxins were running slightly below BSA as expected. 336/69 seemed degraded. The protoxin sample ran at about 130 kilodaltons. I gave Jacqui Leighton one of these toxin samples for injections into rabbits and mice to prepare antibodies. The rest of the toxins were all stored at -70°C for later use. On page 139, I wrote "Protoxin" in the upper left hand corner of the page. I prepared a sample on page 81 and had it

> stored. I ran this protoxin (336/81) on the gel on page 138 and observed a band. When I measured protein amounts by a Biorad assay and then looked at it on a gel for a given protein form, in this case a toxin versus protoxin form, I noticed that the values would differ just due to how the protein folds in solution. I signed page 138 on 10/29/86. Hoffman signed it on 11/10/86. Rocheleau signed it on 10/15/86. I signed pages 136-139 on 10/29/86. Hoffman signed them on 11/10/86. Rocheleau signed them on 10/15/86.

Page 138 of Adang's Notebook 336 (AX 006, 336/138) includes the SDS-PAGE results for Bt crystal proteins (notebook #/page #) 336/136, 336/125, and 336/98, indicating molecular weights less than 66 kilodaltons, and Bt crystal proteins 336/69, 336/106, and 336/81 indicating molecular weights greater than 92 kilodaltons (compare, for example, SDS-PAGE results in Notebook 336, page 100 (AX 006, 336/100)). Page 139 of Adang's Notebook 336 reads (AX 006, 336/139):

> PROTOXIN
>
> > The 336/81 was from 1/19/86. This was stored -70°C. On 6/10 divided into 50λ aliquots; froze in dry ice + stored -20°C. This is good stuff.
> >
> > Note: the toxin band is allways [sic] darker than a given amount of protoxin. One of these is giving an incorrect protein reading w/ the Bradford.

Whether or not the SDS-Page results in notebook 336/139 establish that HD73 proteins 336/69, 336/106, and 336/81 are protoxins running about 130 kilodaltons, the relationship between HD73

proteins 336/69, 336/106, and 336/81 and the Bt crystal protein gene insert of the pH450 construct used to transform UC82 tomato plant cells or tomato plants regenerated therefrom has not been explained or established.

AD-1479 is a copy of page 41 of the Declaration Under 37 C.F.R. §1.672(b) by Sara E. (Tatchell) Patterson, dated June 30, 1997. Pages 41-42 of Sara E. (Tatchell) Patterson's declaration are reproduced below (AD-1479-1480):

> d. Western Blot No. 1: Detection of B.t. Protein in Tomato Plants Transformed With pH450
>
> Western blots were performed to assay for the presence of the B.t. insecticidal crystal protein in UC82 transgenics. The protein samples in this test were prepared from plant tissue of transgenic lines that originated from pH450 hypocotyl transformation initiated on February 2, 1985, (AB 55/71). In particular, protein samples from the R_0 transgenic line L-7, transformed with pH450, were assayed by immunodetection of the B.t. protein on a Western blot of these samples.
>
> The Western blot was performed according to a standard protocol described in Part III, subsection 8, of this declaration, and detailed below according to the date the various steps of the protocol were performed.
>
> (1) On April 13, 1987, (ED1304/31A) protein samples were prepared from plant tissue using a standard protocol involving TCA precipitation. On this same day, the protein samples were loaded and resolved by electrophoresis on a protein gel. In addition, the identity of each sample was recorded.

On April 13, 1987, (ED1304/31A) the protein samples resolved in the gel, were transferred to nitrocellulose membrane, blocked with nonfat dried milk, and then the membrane was hybridized overnight with two different rabbit antibodies, R16 and R118, that specifically bind to B.t. protein.

Also on April 13, 1987, (ED 1304/32A) the protein remaining in the gel, after transfer, was stained with Comassie Blue overnight to verify complete transfer of proteins.

(2) On April 14, 1987, (ED 1304/32A) the membranes were washed and incubated with secondary alkaline phosphatase goat anti-rabbit antibodies. Thereafter, the membranes were washed and treated with BCIP/NBT enzymes in order to visually detect bound antibodies indication the presence of B.t.

(3) On April 15, 1987, (ED1304/32A, 32B) results of the Western blot were recorded and the blots pasted to the notebook page (ED 1304/31B). The results of the Western blot assay demonstrated immunodetection of the full-length B.t. insecticidal crystal protein in UC82 transgenic line L-7 transformed with pH450. See Figure 3, attached.

(4) On April 16, 1987, I sent to Adang et al. photographs of Western blots of protein from the transgenic line L-7 transformed with pH450. In addition, I enclosed a letter, along with the photos, detailing the protocol used for the Western blot analysis.

(5) On April 20, 1987, Adang wrote a letter to me, which I received acknowledging receipt of the letter and photos that I sent to him on April 16, 1987. In this letter, he stated the following about the photographs:

> Thank-you for sending the western photo, it looks very encouraging. It is a good idea to run protoxin for you BT standard. The TCA precipitation followed by the ammonium sulfate cut gave the cleanest lanes, however, it will be important to

> compare the 133 kD prototoxin with the different extraction protocols to see which is best. Also, do you know the amount of plant protein loaded per lane?
>
> Sincerely,
>
> Mike Adang p.s. Joshua Lederberg saw your western.

ED-1304/31A, ED-1304/31B, ED-1304/32A, and ED-1304/32B cited in the Declaration Under 37 C.F.R. §1.672(b) By Sara E. (Tatchell) Patterson, dated June 30, 1997 (AD 1479-1480), refer to pages 31A, 31B, 32A, and 32B of Elizabeth Dale's Laboratory Notebook No. 1304 (AX 81). Page 31A of ED-1304 is entitled "SDS PAGE & Western ~ tissue prep'd #13A25, diff. methods (L-7)" for Project No. 13A31, signed April 15, 1987, by Elizabeth Dale, and signed April 18, 1987, as disclosed to and understood, by Sara Tatchell (AX 81). The top of page 31A of ED-1304 signed April 15, 1987, by Elizabeth Dale, and signed April 18, 1987, as disclosed to, and understood by, Sara Tatchell, defines the purpose of Project No. 13A31 (AX 81):

> Purpose: To test for Bt presence in 'transformed' UC82, L-7, tissues prep'd in exp#13A25. L-7 and UC82 control tissue samples prepared by the methods found to give best results in exp# 13A29 (u-Bt samples tested) will be used.

Bridging pages 32A and 32B of ED-1304, signed April 16, 1987, by Elizabeth Dale, and signed April 18, 1987, as disclosed to

and understood, by Sara Tatchell, are the results of Project No. 13A31 (AX 81):

> Results: Bands w/ higher MW than Bt toxin were visible in the L-7 tissues of all three methods.
> L-7 prepared via 1b-a gave single band; UC82 control prep'd same way was clear of this band
> L-7 prep'd via 1b gave a single band at a slightly higher MW than the band in 1b-a; UC82 control via 1b was clear
> L-7 prep'd via 4 had two bands -- one which migrated to the same place as the single band in L-7, 1b-a, and the other at the same place as the L-7, 1b band. UC82,4 control lanes on gel #2 were clear, but on gel #1 there were faint double bands which may be due to over flow from lane 15.
> With the exception of L-7,4 which had a slightly stained area corresponding to Bt toxin std migration (see photo of 'wet' nitrocellulose), L-7 tissues did <u>not</u> indicate the presence of Bt in the toxin form, MW-60kD; however it may be that the bands observed in L-7 are Bt protoxin. Next: Run protoxin std w/ L-7 & UC82 tissue preps.

Pages 33 and 35 of ED-1304, signed April 21, 1987, by Elizabeth Dale, and signed April 24, 1987, as disclosed to and understood, by Sara Tatchell, are respectively, the Purpose and the Results of Project No. 13A33, entitled "SDS Page & Western ~ Bt protoxin with L-7 & UC82" (AX 81)(* means illegible)(emphasis added):

> Purpose: Western (#13A31) on 'transformed' UC82, L-7, tissue showed protein bands w/ high MW (vs. Bt toxin) close to where Bt protoxin would migrate. As a check on this, samples to be run

for Western will include freshly solubilized crystal Bt protoxin (HD73, 3-21) along w/ the L-7 & UC82 control tissue extracts (prep'd #13A25; 13A31, run BRL PM as usual & * moth blood for MW check. [(ED-1304/33);]

Results: -Protoxin stds came out very clearly, 25 ng protox. was easily detectable in tissue extract (UC82) on the nitrocel filter, #1, that was incubated w/ both R118+R116αBt; on filter #2 the band was faint but detectable (incubated R118αBt only).

-Bands in L-7 sample migrated to very near where Bt protox stds migrated. As in 13A31, L-7 tissue extract prep'd via 4 gave double band; prep'd via 1b gave single band corresponding to upper band of 4; & prep'd via 1b-a gave single band at same place as lower band of 4.

-Protoxin stds (133 kD) and lower band of L-7, 4 along w/ single band of L-7,1-b* migrated to ~ the same place, thus Bt protoxin in L-7 tissue.

-(Upper band of 4 & single band of 1-b) Lanes 10, 12, & 13 and slightly *6 on filter #2, UC82 controls, had the upper band as well. Perhaps this protein (upper band) which is cross-reacting w/ the anti-bodies was produced in the plant in response to something which did not occur in the control tissue against which the antibodies were cleared. These bands were not as visible on filter #1 however. [(ED-1304/33).]

Adang ultimately argues (AB 18):

On October 23-29, 1986, Dr. Binns initiated additional bioassays of the transformed tomato plants from the pH450 hypocotyl transformation initiated on February 2, 1985. (AD-1486-1487, citing AX-025, Notebook 1188, p. 71) These bioassays were designed to test for B.t. insecticidal activity against Manduca sexta, the tobacco hornworm. The bioassays indicated that the B.t. protein was insecticidal in the transformed tomato plants. These results were

reported in Table 13 of Adang's 1988 application. (AD-1486) This constituted another Adang actual reduction to practice of tomato plants within the scope of the Count before Fischhoff's November 1986 filing date.

AD-1486-1487 to which Adang's Brief refers (AB 18), are pages 48-49 of the Declaration Under 37 C.F.R. §1.672(b) by Sara E. (Tatchell) Patterson, dated June 30, 1997. To be complete, pages 48-50 of Sara E. (Tatchell) Patterson's declaration are reproduced below (AD-1486-1488):

a. Manduca Sexta Bioassay of Tomato Plants Transformed With pH450 and R3-11

R_0 and R_1 transgenic UC82 tomato plants carrying the full-length B.t. gene were tested for insecticidal activity using Manduca sexta in a bioassay. The UC82 transgenics tested in this assay originated from: 1) the pH450 hypocotyl transformation initiated on February 2, 1985, (AB 55/71); and 2) the R3-11 hypocotyl transformations initiated on November 29, 1984 (AB 55/68). Leaf samples and whole plants from the pH450 and R3-11 transformed lines were assayed.

Additional bioassays of the UC82 transgenics originating from the same pH450 transformation initiated on February 2, 1985, (AB 55/71) were performed on the following dates and recorded:

October 23, 1986, (ST 1188/1)

July 9, 1987, (ST 1188/22)

November 9, 1987, (1188/32)

February 20, 1988, (1188/34).

The bioassay was performed according to a standard protocol described in Part III, subsection 10, of this declaration, and detailed below according to the date the steps of the protocol were performed.

(1) On October 23, 1986, (ST 1188/1) eggs of tobacco hornworms, Manduca sexta, were received from Carolina Biological Supply.

(2) On October 24, 1986 (ST 1188/1) the newly hatched Manduca sexta were placed on moistened filter paper in petri plates with leaf samples taken from the UC82 transgenic lines transformed with pH450 or R3-11. See Figures 4A and 5A, attached.

Also on October 24, 1986 (ST 1188/1) the newly hatched Manduca sexta larva were placed on whole plants of the UC82 transgenic lines transformed with pH450 or R3-11.

(3) From October 24 through 30, 1986, (ST 1188/2A, 2B, 3A, 3B, 3C) the leaf samples assayed with Manduca sexta were observed daily and photographed. The observations of the leaf samples and whole plants were recorded.

(4) On October 27, 1986, (ST 1188/1) color slides of the leaf samples assayed were taken and are attached as Figures 4A and 5A.

(5) From October 24 through 30, 1986, (ST 1188/2B, 3B) a specific list of the leaf sample and whole plant lines, along with daily observations concerning the bioassay of each, were recorded. The leaf sample and whole plant lines on this list originated from UC82 transgenics. Wild type UC82 transgenic lines tested and recorded on this list originated from the pH450 transformation initiated on February 2, 1985 (AB 55/71) except transgenic line 9CG0201 which is an R_0 plant from the R3-11 transformation initiated November 29, 1984 (AB 55/68).

(6) On October 29, 1986, (ST 1188/2B) the results of the bioassay for some of the lines were summarized and recorded. The summary of these results indicated that the mortality of the Manduca sexta placed on the pH450 transformed leaf samples was: 64 % for L-7, 75 % for line 4-2e, 62.5 % for line 4-2f, 40 % for line 1-1A, and 17 % for line 2-3A. The mortality of the Manduca sexta placed on the R3-11 transformed leaf sample from line 9CG-2 was variable: on plates the mortality was 40 % and on whole plants the mortality was 80 %. However, if the average weight of the surviving Manduca placed on the transgenic leaf samples, then these results indicate that all the transgenic lines assayed exhibited a reduction in the weight of the Manduca.

Also on October 29, 1986, (ST 1188/3A) I made a graph, titled "Bioassay #1", summarizing the results for this assay. This graph was compiled from data recorded October 24 through 29, 1986 (ST 1188/2B, 3B).

Pages 1 and 2B of Sara Tatchell's Laboratory Notebook No. 1188 appear to have been signed by S. Tatchell on October 26, 1986, and signed on November 5, 1986, as disclosed to and understood by, E. Dale. Pages 3A-3C appear to have been signed by S. Tatchell on October 29, 1986, and signed on November 5, 1986, as disclosed to and understood by, E. Dale (AX 81, Book No. 1188, pp. 1-3). Page 1 of Sara Tatchell's Laboratory Notebook 1188 includes the following handwritten entries (AX 81, Book No. 1188, p. 1)(* = illegible):

TITLE Bioassays of 'UC82' Lycopersicum esculentum with Manduca sexta tobacco hornworm for Bt protein PROJECT NO. 9A1 BOOK NO. 1188

Interference 103,324

PURPOSE: To determine if putative Bt protein containing R_0 and R_1 plants transformed with pH450 (exp. 55A71) are resistant or sensitive to Manduca sexta (Tobacco hornworm were ordered from Carolina Biological Supply).

PROTOCOL: 1) R_0 and R_1 plants have been isolated from normal greenhouse facilities to avoid greenhouse spraying.
2) Eggs are ordered from Carolina Biological Supply and placed on plants and leaf discs. (Both whole plants & leaf discs on filter paper assayed)
Each plant assayed receives 5 hornworms/plant
plates receive $\geq$5 hornworms/plate
#s recorded

10/23/86
Eggs received

10/24/86
Eggs placed with plant material (early morning)
Some eggs beginning to hatch Mid -> late afternoon - evening some worms observed dead
1 on IC82 leaf
1 on 55PH-71-4-***
All plates quantified at this point-# worms/plate

10/25/86

10/26/86 Photographs taken of all plates/observations recorded.
food supply plate #1 and #4 quite depleted

Photograph negatives on Pages 2A and 3C of ST1188 are illegibly dark. Pages 2B and 3B present handwritten data. The summary of "# alive" and "% fatality" on page 2B of ST 1188 is reproduced below (AX 81, ST 1188/2B):

Interference 103,324

Plant material	# worms/plate	# alive/today	% Fatality
UC82	33	25/33	24
L-7	14	6/14	64%
1-1A	5	3/5	40%
4-2e	4	1/4	75%
4-2f	8	3/8	62.5
2-3A	6	5/6	17

Page 3A presents the following type written "Data from 'Bio assay #1'" signed by S. Tatchell on October 29, 1986, and E. Dale on November 5, 1986, showing the comparative weights and relative plate %s survival of Manduca sexta placed on tomato leaf samples taken from untransformed tomato plants (UC82 (AD 1487-88)), tomato plants regenerated from tomato plant cells transformed by R3-11 (9CG-2 (AD 1487-88)), and tomato plants regenerated from tomato plant cells transformed by pH450 (L-7; 4-2f; 4-2e; 1-1a; 2-3a (AD 1487-88)):

Variety	Worm Weight (mg)-1	On Plates % Survival-1[(#worms)
UC82	29.4	79 (33)
9CG-2	21.5	60 (5)
L-7	.5	36 (14)
4-2f	9.5	37.5(8)
4-2e	9.17	25 (4)
1-1a	5.875	60 (5)
2-3a	20	83 (6)

"[E]xp. 55A71" which Sara [Patterson] Tatchell cites on page 1 of Sara Tatchell's Laboratory Notebook No. 1188, appears to refer to the report of experiments performed by A. Binns

in Laboratory Notebook No. 55, page 71, (AB 55/71); the first half signed by Andrew N. Binns on May 24, 1985, and Sara Piddington on October 1, 1985 (AX 81); the second half signed by A. Binns on July 18, 1985, and Sara Piddington on October 1, 1985 (AX 81). AB 55/71 reports the following information (AX 81)(* means illegible):

Transformation of UC82
hypocotyl segments by
LBA4404(pAN/Kan) & LBA 4404(pH450)

Purpose: Madison Labs have constructed pH450 which is their micro Ti carrying the B.t. crystal protein as well as ocs & Kan^R. Here we will transform hypocotyl segments with this strain (LBA4404 (PH450)) and the same strain without the crystal protein gene. LBA4404 (pAN/Kan)

Protocol

(1) cut UC82 hypocotyl segments into ~ 7 mm segments (seeds germinated 2-6-85)

(2) inocculate basal and with (< pH450 or (pAN/Kan overnight cultivating on L plates & selective antibodies chloramphen 50 strep 250

4/2/85 (3) place, basal end up, into R3B plates

4/4/85 (4) after hrs transfer segments to R3B plates plus 500/200 C**

4/18/85 (5) when wound callus develops, transfer to either 0 or 5 µg/ml G418 & ***;

cut wound callus in upper (u) and lower (L) half; test each independently

(6) after tissues grow up, test on R3G & 0,10,20μg/ml G418 (20 pieces/plate)

			0	10	20
5/1/85	Results from: pH450	U/0	+++	+1/2	±
		U/5	+++	±	±
		L/0	+++	±	±
		L/0	+++	±	-
	from pAN/Kan	U/0	+++	+1/2	±
		U/5	+++	+	±
		L/0	+++	+	±
		L/5	+++	±	±

5/24/85 (7) transferred to R3G & G418 (μg/ml)

	from:		0	10	20	30
20-25 pieces re*treatment	pH450U	0->0	+++	+	3/20+ 17/20-	-
	pH450U	0->10	++	+	-	-
	pH450U	5->0	+++	+	-	21/23- 2/23+?
	pH450L	5->0	+++	+	23/25- 2/25+?	-
	pAN/KanU	5->10	+++	+	12/14- 2/14+?	-
Recorded 6/28/85	pAN/KanL	5->10	+++	+	-	-
	pAN/KanU	0->10	++	+	16/19- 3/19+?	16/19- 3/19+?
	pAN/KanL	0->10	++	+	-	-

7/1/85 (8) pieces selected from 10, 20, 30 μg/ml G418 that appear to be growing and placed onto R3 3C5ZR + 20 μg/ml G418 to test for regeneration

7/18/85 (9) 4 pieces clearly growing: pH450U from 0->0->20
pH450L from 5->0->10
(2 pieces each)
then ** were split and placed the vials of R3 3C5ZR ± 20 μg/ml G418 +250/50 μg/ml L/U

** labeled pH450 U-1
pH450 U-2
pH450 L-1
pH450 L-2

Also enough tissue from U-1, L-1, L-2 to run oct test
incubate overnight on R3G + ***
results oct assay: U-1 +, L-1 weak, L-2 +

(10)[.]

Having considered all the evidence of record, we find that the preponderance of the evidence supports Adang's argument that it regenerated a tomato plant from a tomato plant cell transformed to comprise a full length Bt crystal protein gene which is capable of encoding Bt crystal protein of 130 kD under control of a promoter which directs expression of said structural gene in said tomato plant by June 12, 1986. However, the preponderance of the evidence of record does not establish that Adang reduced an invention defined by the interference count to practice on June 12, 1986. The evidence does not establish that on June 12, 1986, Adang recognized and appreciated that the Bt crystal protein gene is expressible in said regenerated tomato plant in amounts which destroy or control Lepidopteran insects. The evidence establishes that the ability of the regenerated tomato plants to control Lepidopteran insects was not recognized and appreciated until October 29, 1986. On that date Adang

recognized and appreciated that tomato plants had been prepared that expressed some form of the Bt protein in amounts which were insecticidal to lepidopteran insects. In other words, by October 29, 1986, the inventors recognized and appreciated that they had successfully achieved the goal of creating tomato plants which were rendered insecticidal by expressing the Bt crystal protein. However, on October 29, 1986, it had not been confirmed that those tomatoes included a full length Bt crystal protein gene capable of expressing a Bt crystal protein of about 130 kD. While Adang testifies to its belief that the tomato plants tested included the full length gene, the evidence shows only that he was informed by others that the plants included the full length gene. However, subsequent Western blot testing confirmed that the insecticidal tomato plants in fact expressed a Bt crystal protein of about 130 kD and, therefore, confirmed that the plants comprised the full length gene that was "capable" of expressing the 130 kD protein. Evidence of subsequent testing may be admitted for the purpose of showing that an embodiment was produced and that it met the limitations of the count. Cooper, 154 F.3d at 1331, 47 USPQ2d at 1904; Silvestri, 496 F.2d at 598, 181 USPQ at 709. This subsequent testing confirmed that by October 29, 1986, Adang had actually reduced to practice an

embodiment meeting all the limitations of the count. Our findings are supported by the following direct evidence and corroborated testimony:

(1) On February 2 and 3, 1984, Drs. John D. Kemp, Michael J. Adang, and Donald J. Merlo of Agrigenetics arranged a collaborative project with Dr. Andrew Binns of the University of Pennsylvania whereby personnel of Dr. Binns' laboratory would use vectors containing B.t. crystal protein genes developed at Agrigenetics to transform tomato plants and perform bioassays evaluating the expression of the B.t. crystal protein genes in tomato plants (AD 528-31; AX 021; AX 119-120; AD 1379; Ad 1382; AD 1444).

(2) On March 22, 1985, Donald J. Merlo delivered to Adang the LBA4404(pH450) construct comprising the Bt crystal protein gene under control of the mannopine synthase promoter with which Dr. Binns began transforming tomato tissue later in 1985. Merlo declared that pH450 comprised a full length Bt crystal protein gene with a coding region of approximately 3.78 kbp (AD 1379-1387 (see especially AD 1382, para. 13a.); and AD 0531);

(3) On March 20, 1986, Sara (Tatchell) Patterson, working for Dr. Binns, determined by ELISA assay using monoclonal antibodies to detect the presence of B.t. crystal protein that

tomato plants regenerated from tomato plant cells transformed with pH450 tested positive for the expression of B.t. crystal protein (AB 17-18; AD 1476-1477; AX 81, ST 985/17, 18; AX 81, two part insert between ST 985/17 and ST 985/18);

(4) On April 4, 1986, (a) Adang filed Adang '86 which reports (i) the expectation that the Bt crystal protein gene in pH450 should transcribe mRNA molecules of about 3.8 kbp (Adang '86, p. 75, last para.) and (ii) the results that tobacco plant tissue regenerated from tobacco plant cells transformed using pH450 produced Bt crystal protein and tested positive for insecticidal activity (Adang '86, pp. 73-75), and (b) Adang and his technician, Thomas Rocheleau (AD 1567-68), had concluded that several tobacco plants had been transformed to contain "Bt/Bam 3.8Kb full-length HD-73 [crystal protein] gene" (AD 1589);

(5) On April 4, 1986, Adang filed Adang '86 which discloses that "full length crystal protein" means crystal protein "protoxin of approximate molecular weight (MW) of 130 kilodaltons (kD)" (Adang '86, pp. 1 and 28);

(6) On June 12, 1986, Dr. Binns delivered tomato plant tissue and seeds to Adang and informed Adang that the tomato plant tissue and seeds had been regenerated from tomato plant cells Dr. Binns transformed beginning in 1985 using a pH450

construct comprising a full length Bt crystal protein gene under control of a mannopine synthase promoter (AB 18; AD 0373, para. 130; Adang Notebook 336, p. 140);

(7) On October 23-29, 1986, Sara (Tatchell) Patterson, working for Dr. Binns, performed bioassays designed to test for B.t. insecticidal activity against Manduca sexta, the tobacco hornworm, on tomato plants regenerated from UC82 tomato plant cells transformed by Dr. Binns using pH450 (AB 18; AD 1486-1488; AX 25, ST 1188/1-3; AX 81, ST 1188/1-3);

(8) On October 29, 1986, Sara (Tatchell) Patterson summarized and recorded increased mortality and reduced weight of Manduca sexta (Lepidopteran insects, Adang '86, Table 1, p. 83) for bioassays designed to determine the B.t. insecticidal activity against Manduca sexta of tomato plants regenerated from UC82 tomato plant cells transformed by Dr. Binns using pH450 in 1985 (AD 1487-1488; AX 81, ST 1188/2B, 3A, and 3B);

(9) Neither the arguments in Adang's brief at final hearing (AB 18), the Declaration of Michael J. Adang, dated July 1, 1997 (AD 0373, para. 130), nor page 140 of Adang's Notebook No. 336 (AX 006), establish that prior to October 29, 1986, "Sara Tatchell had done some experiments with . . . [tomato tissues regenerated from tomato plant cells transformed with pH450] where

she was feeding them to worms" and "was seeing some insect toxicity" (AD 0373);

(10) On April 15-16, 1987, E. Dale signed Notebook No. 1304, pages 31B, 32A, and 32B (signed April 18, 1987, as disclosed to, and understood by, Sara (Tatchell) Patterson), which record Western blots and the following written results for Project 13A31, entitled "SDS PAGE & Western ~ tissue prep'd #13A25, diff. methods (L-7)" (AX 81, ED 1304/31A) "[t]o test for Bt presence in 'transformed' UC82, L-7, tissues prep'd in exp#13A25" (AX 81, ED 1304/31A)(AX 81, ED 1304/32A and 32B)(emphasis added):

> Results: Bands w/ higher MW than Bt toxin were visible in the L-7 tissues of all three methods.
> L-7 prepared via 1b-a gave single band; UC82 control prep'd same way was clear of this band
> L-7 prep'd via 1b gave a single band at a slightly higher MW than the band in 1b-a; UC82 control via 1b was clear
> L-7 prep'd via 4 had two bands -- one which migrated to the same place as the single band in L-7, 1b-a, and the other at the same place as the L-7, 1b band. UC82,4 control lanes on gel #2 were clear, but on gel #1 there were faint double bands which may be due to over flow from lane 15.
> With the exception of L-7,4 which had a slightly stained area corresponding to Bt toxin std migration (see photo of 'wet' nitrocellulose), L-7 tissues did not indicate the presence of Bt in the toxin form, MW-60kD; however it may be that the bands observed in L-7 are Bt protoxin. Next: Run protoxin std w/ L-7 & UC82 tissue preps.

(11) On April 21, 1987, Elizabeth Dale signed Notebook No. 1304, pages 33 and 35 (signed April 24, 1987, as disclosed to, and understood by, Sara (Tatchell) Patterson), which record the following written results for Project 13A33, entitled "SDS Page & Western ~ Bt protoxin with L-7 & UC82" (AX 81, ED 1304/33) "[a]s a check . . . [Project 13A31 with] samples to be run for Western . . . includ[ing] . . . freshly solubilized crystal Bt protoxin (HD73, 3-21) along w/ the L-7 & UC82 control tissue extracts (prep'd #13A25; 13A31" (AX 81, ED 1304/33)(AX 81, ED 1304/35)(emphasis added):

Results: -Protoxin stds came out very clearly, 25 ng protox. was easily detectable in tissue extract (UC82) on the nitrocel filter, #1, that was incubated w/ both R118+R116αBt; on filter #2 the band was faint but detectable (incubated R118αBt only).
-Bands in L-7 sample migrated to very near where Bt protox stds migrated. As in 13A31, L-7 tissue extract prep'd via 4 gave double band; prep'd via 1b gave single band corresponding to upper band of 4; & prep'd via 1b-a gave single band at same place as lower band of 4.
-Protoxin stds (133 kD) and lower band of L-7, 4 along w/ single band of L-7,1-b* migrated to ~ the same place, thus Bt protoxin in L-7 tissue.
-(Upper band of 4 & single band of 1-b) Lanes 10, 12, & 13 and slightly *6 on filter #2, UC82 controls, had the upper band as well. Perhaps this protein (upper band) which is cross-reacting w/ the anti-bodies was produced in the plant in response to something which did

> not occur in the control tissue against which the antibodies were cleared. These bands were not as visible on filter #1 however. [(ED-1304/33).]

(12) A comparison of the April 21, 1987, Western blot migrations of Bt crystal protein extracted from L-7 tomato plants regenerated from UC82 tomato plant cells transformed using pH450 and Bt crystal protein in protoxin standards (Bt crystal protein of about 133kD) showed that L-7 tomato plants expressed the same full length Bt crystal protein gene of about 130 kD;

(13) On April 21, 1987, Sara (Tatchell) Patterson confirmed that pH450 did in fact comprise a full length Bt crystal protein gene which encoded a Bt crystal protein of about 130 kD (AD 1480) (emphasis added):

> (3) On April 15, 1987, (ED1304/32A, 32B) results of the Western blot were recorded and the blots pasted to the notebook page (ED 1304/31B). The results of the Western blot assay demonstrated immunodetection of the full-length B.t. insecticidal crystal protein in UC82 transgenic line L-7 transformed with pH450. See Figure 3, attached.

(14) After the November 20, 1986, filing date of Fischhoff '86, Adang confirmed that tomato plants which had been regenerated from UC82 tomato plant cells transformed using pH450 and had tested positive for toxicity to Lepidopteran insects on

October 29, 1986, comprised a full length Bt crystal protein gene capable of encoding a Bt crystal protein of about 130 kD.

The preponderance of the evidence of record establishes that Adang first actually reduced an embodiment encompassed by Count 1 of this interference to practice on October 29, 1986. Having concluded that Junior Party Adang first reduced an embodiment encompassed by Count 1 of this interference to practice on October 29, 1986, i.e., prior to the November 20, 1986, filing date of Fischhoff '86, we proceed to consider Fischhoff's case for priority of the invention defined by the count.

2. Fischhoff's case for priority of invention

In its decision entered September 29, 2000, the Board held (Paper No. 233, p. 19):

> [T]omato plants encompassed by Count 1 (1) must have been regenerated from a tomato plant cell transformed by a full length Bt crystal protein gene which encodes Bt crystal protein of about 130 kD under control of a promoter which directs expression of said structural gene in said tomato plant cell, and (2) must produce amounts of Bt crystal protein protoxin of about 130 kD which destroy or control Lepidopteran insects in any way.

Because of its narrow interpretation of the scope of the invention defined by Count 1, the Board held that Adang had not actually reduced an embodiment of the invention encompassed by

Count 1 to practice prior to the November 20, 1986, filing date of Fischhoff '86. Now, having considered Adang's evidence in light of the broader interpretation of the scope of the invention defined by Count 1 by our reviewing court in Adang, 286 F.3d at 1355, 62 USPQ2d at 1510, we conclude that the earliest date Adang actually reduced an embodiment of Count 1 to practice was October 29, 1986, i.e., prior to the November 20, 1986, filing date of Fischhoff '86. Hence, we proceed to consider for the first time Fischhoff's evidence that it actually reduced an embodiment of Count 1 to practice prior to Adang.

A. Precedent

We have revisited the precedent cited in Part A of Adang's case for priority of invention. In particular, we note the teachings of Schendel v. Curtis, 83 F.3d 1399, 38 USPQ2d 1743 (Fed Cir. 1996), Cooper v. Goldfarb, 154 F.3d 1321, 47 USPQ2d 1896 (Fed. Cir. 1998), and Loral Fairchild Corp. v. Matsushita Electrical Industrial Co., 266 F.3d 1358, 60 USPQ2d 1361 (Fed. Cir. 2001), as we consider Fischhoff's case for priority of invention. These cases instruct that an actual reduction to practice requires proof of a physical embodiment meeting every limitation of the count and knowledge that it worked as intended.

Accordingly, Fischhoff must establish that he regenerated a tomato plant from a "tomato plant cell transformed to comprise a full length Bt crystal protein gene capable of encoding a Bt crystal protein of about 130 kD under control of a promoter which directs expression of said structural gene in said tomato plant cell" and the regenerated tomato plant "produce[d] amounts of a Bt crystal protein of any size which destroy or control Lepidopteran insects in any way." Adang, 286 F.3d at 1355, 62 USPQ2d at 1510.

Testimony of an actual reduction to practice by an inventor must be corroborated. Loral Fairchild Corp. v. Matsushita Electrical Industrial Co., 266 F.3d at 1363, 60 USPQ2d at 1365, instructs:

> "In order to establish an actual reduction to practice, an inventor's testimony must be corroborated by independent evidence." Cooper v. Goldfarb, 154 F.3d 1321, 1330, 47 USPQ2d 1896, 1903 (Fed. Cir. 1998). The sufficiency of such corroborating evidence is evaluated under a "rule of reason," considering all of the pertinent evidence. Id. ("The rule requires evaluation of all pertinent evidence when determining the credibility of an inventor's testimony. . . . In order to corroborate a reduction to practice, it is not necessary to produce an actual over-the-shoulder observer. Rather, sufficient circumstantial evidence of an independent nature can satisfy the corroboration requirement.").

Most pertinent to this case is the following statement in Loral Fairchild Corp. v. Matsushita Electrical Industrial Co., 266 F.3d at 1364, 60 USPQ2d at 1365-66:

> In Cooper, 154 F.3d at 1330, 47 USPQ2d at 1903, we held that reduction to practice had been proven by the inventor's testimony corroborated by testimony of his co-workers that he told them of his reduction to practice, and that they had seen him obtain materials of the type necessary to practice the invention. We so held even though "no direct evidence supported Goldfarb's testimony that he measured fibril length and observed tissue ingrowth." Id. ("We agree with the Board that circumstantial evidence provided sufficient corroboration."); see also Lacotte v. Thomas, 758 F.2d 611, 613, 225 USPQ 633, 634 (Fed. Cir. 1985) (affirming finding of reduction to practice, stating "[t]he testimony of Thomas, the inventor, and the written evidence of his reduction to practice in his notebook, are corroborated by independent circumstantial evidence of his withdrawal of supplies to practice the invention, as well as independent corroborating testimony of his associate, Lee.").

B. Evidence and findings

The Declaration of Fischhoff and Rogers, dated October 4, 1996 (Fischhoff Record, pages 1-15 (FR 1-15)), states (FR 8):

> On September 11, 1985 a full-length B.t. gene clone in plant transformation vector pMON316 was identified and named pMAP1204. See Bowdish notebook page 3064996 of that date. (Exhibit 21 [(F-RX 21)]).

Fischhoff-Rogers Exhibit 21 cited therein is handwritten Monsanto Co. notebook No. 3064996, signed September 11, 1985, by Kathy Bowdish, and signed January 26, 1986, as read and understood by

Linda Zurfluh (F-RX 21). The "Subject" of No. 3064996 is "Orientation digests for Aha term. Bt in pMON316" (F-RX 21). What express references there are to pMON316 and pMAP1204 in Fischhoff-Rogers Exhibit 21 appear in its lower half (F-RX 21) (* means unclear):

```
0.6%Agarose          Results:
(1) 316 uncut        #4, 6, 12, 15 are (+) orientation
(2) 316/Kpn          #5, #7 are (-) orientation
(3) #3 uncut         other two are strange.
(4) #3/
(5) #4 <-----chose this to streak out on * & grow up for
(6) #5       larger culture to make prep & isolate fragment
(7) #6       to clone into E. coli expression vector.
(8) #7       =pMAP1204
(9) #9
(10)#12      9/17 frozen away
(11)#15
(12)λHindIII[.]
```

The Declaration of Katherine S. Bowdish dated October 4, 1996 (FR 16-23), states (FR 22):

> On September 11, 1985, a full-length B.t. gene clone in plant transformation vector pMON316 was identified and named pMAP1204. See Bowdish notebook page 3064996 of that date (Exhibit 19).

Katherine S. Bowdish Exhibit 19 is substantially identical to Fischhoff-Rogers Exhibit 21.

At this point, we look to the evidence of record that a full length Bt gene capable of encoding a Bt crystal protein of about 130 kD was in fact present in the plant transformation construct

identified and named pMAP1204. At page 15, line 1, to page 16, line 25, of both Fischhoff's parent U.S. Application 06/932,818, filed November 20, 1986 (Fischhoff '86), and Fischhoff's continuing involved U.S. Application 07/813,250, filed December 23, 1991 (Fischhoff '91), we find the following disclosure:

> Referring to Figure 11, plasmid pMON316, a derivative of pMON200 (Fraley et al. 1985; Rogers et al. 1985) is a co-integrating type intermediate vector which contains the CaMV35S promoter and the 3' polyadenylation signal of the NOS gene. pMON316 has unique cleavage sites for the restriction endonucleases BglII, ClaI, KpnI, XhoI and EcoRI located between the 5' leader and the 3' NOS polyadenylation signals. The cleavage sites provide for the insertion of the coding sequences carrying their own translational initiation signals immediately adjacent to the CaMV35S leader sequence. Plasmid pMON316 retains all the properties of pMON200 including spectinomycin resistance for selection in E. coli and A. tumefaciens as well as a chimeric kanamycin gene (NOS/NPTII/NOS) for selection of transformed plant tissue and the nopaline synthase gene for ready scoring of transformants and inheritance in progency.
>
> Referring to Figure 5, a plasmid was constructed for the expression of the B.t. toxin gene in plants by ligating the 4.5 kb BamHI fragment containing the toxin gene from pMON294 into pMON316 which had been digested with BglII. A plasmid which contained the toxin gene oriented such that the translational initiator was adjacent to the CaMV35S promoter was identified by digestion with EcoRI and designated pMON8053. Another chimeric plant gene was prepared comprising the full-length construct in which the structural coding sequence for the B.t. toxin was truncated at the DraI site at position 3479. This site is 10 nucleotides beyond the

translational terminator codon for the coding sequence for the full-length B.t. toxin. Thus, this construct contains the full-length coding sequence but very little 3' flanking sequence from the B.t. subspecies kurstaki gene. This construct is designated pMON9712.

Referring to Figure 6, plasmid pMON9712 was prepared by digesting pMON294 with endonuclease DraI. A pair of complementary oligonucleotides having the following sequence were synthesized:

5'-TAGTAGGTAGCTAGCCA-3'
3'-ATCATCCATCGATCGGTCTAG-5'

When annealed to one another these oligonucleotides encode translational terminators in all three reading frames. The annealed oligonucleotide pair is flush-ended at one end and provides a four nucleotide single-stranded region capable of ligation to BglII digested DNA at the other end. The oligonucleotides were annealed to one another and ligated to pMON294 DNA which had been digested with DraI. The ligated DNA was digested with BglII, and a BglII fragment of approximately 3.5 kb containing the desired B.t. toxin coding sequence was isolated. This fragment extends from the BglII site just upstream of the translational initiation codon to the BglII site created by the oligonucleotide pair. This BglII fragment was ligated with BglII digested pMON316. A clone (pMON9712) was identified in which the translational initiator for the toxin gene was adjacent to the 35S promoter by digestion with EcoRI.

We utilize the teachings of Fischhoff '86 and '91 solely to interpret information previously reported in the Fischhoff-Rogers/Bowdish declarations and exhibits.

Bowdish's notebook No. 2916287 signed April 4, 1985, entitled "T419 * truncated B.t. -> checking DNA," reports that "T419 DNA (=pMON294#12)" (F-RX 12/BX 9). Bowdish's notebook

No. 3064935 signed June 17, 1985, entitled "Checking sites for truncated B.t.'s," reads (F-RX 13/BX 10 (excluding drawings)):

T419 = pMON294

Places for termination:

HaeIII	Check for sites in pMON294 -> add linkers to T419 DNA
HindIII	Check for toxicity, size of protein in pMON5528
BclI	(As Bgl fragment)
ScaI	Bgl fragment into pMON316 and/or binary vector
AhaIII(DraI)	

pMON146 = precursor to pMON294

HaeIII in T419: check for HaeIII sites in vector:
If no sites in vector, should get 850 & 150bp fragment. Largest piece w/ Bt must be at least 1500bp + more to next HaeIII site -> from gel: largest fragment should be purified.

HindIII -> should be none in T419, 2 in Bt
~1Kb fragment released -> checks out OK on gel purity large fragment

ScaI — Only one site in Bt - any in 294? There must be one, but Bt fragment must be at least 3373bp long Δ keep large fragment

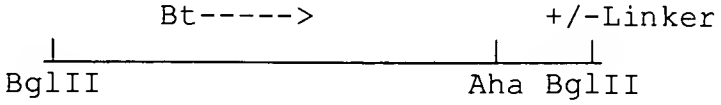
AhaIII/DraI — 2 sites in Bt to release ~ 150bp fragment -> any in 294? must be ~ 3 more, but Bt fragment has to be at least 3470bp long, Δ purify large fragment

From the evidence above, we find that: (1) T419 is another name for the pMON294 vector; and (2) the pMON294 vector comprises Bt

DNA which, when cut by AhaIII/DraI, is a truncated Bt DNA fragment at least 3470bp in length.

Bowdish's notebook No. 3064949 signed July 17, 1985, entitled "Truncated Bt at AhaIII(DraI) site w/ existing DNA's" (emphasis added), reads (F-RX 17/BX 15)(* = unclear)(emphasis added):

> Want to do one truncated Bt w/all existing DNA's (ie - not new preps) and w/new linkers (blunt end) to make sure everything works
>
> Plans:
>
> I. Cut 1µg T419 DNA w/DraI
> Run check gel for complete digest
> Run prep gel -> large fragment onto DEAE membrane
> -> run some * on check gel.
>
> II. Kinase Linkers)
> Anneal Linkers)
> Ligate 14°C on
> Phenol, EtOH, wash, dry
>
> Cut w/ BglII & purify correct fragment onto DEAE (~3.6kb)
>
> => need size markers!



> Clone into pMON5528 as Bgl fragment (Ligate 14°C few hours)
> \>which has been cut w/BglII & phosphatased.
> Check for toxicity, size of protein
>
> Then cut out a clone into pMON316/BglII, phosphatased
> pMON530/ " "

Bowdish's notebook No. 3064949 records a plan to truncate Bt by cutting T419/pMON294 with AhaIII/DraI and to ligate the truncated Bt to the pMON316 transformation vector at common Bg1II sites.

Bowdish's notebook Nos. 3064950 (F-RX 18/BX 16) and 3064951 (F-RX 19/BX 17), wherein Kathy Bowdish records "Truncated Bt at DraI Site Fragment isolation" and "DraI truncated Bt (Isolating fragment)," were respectively signed by Kathy Bowdish on July 17, 1985, and July 19, 1985, and signed as read and understood by Linda Zurfluh on January 26, 1986. Continuing to record work related to the last entry in Bowdish's notebook No. 3064951 (F-RX 19/BX 17), Bowdish's notebook No. 3064967 (F-RX 20/BX 18), entitled "DraI truncated Bt Kinasing Linkers + Ligation to T419/DraI," signed by Kathy Bowdish on August 5, 1985, and signed as read and understood by Linda Zurfluh, on January 26, 1986, describes the ligation of "Linkers to T419/DraI" (F-RX 20).

Although we have studied the evidence cited in the foregoing Fischhoff-Rogers and Bowdish declarations and exhibits, it is not clear from the foregoing evidence how pMAP1204, which was selected for further work at the bottom of Bowdish's notebook No. 3064996 (entitled "Orientation digests for Aha term. Bt in pMON316," signed by Kathy Bowdish on September 11, 1985, and signed as read and understood by Linda Zurfluh on January 1,

1986)(F-RX 21/ BX 19), relates to the AhaIII/DraI truncated Bt fragment Bowdish identified as T419/DraI. The relationship is critical to Fischhoff's case for priority, because Fischhoff-Rogers declares (FR 7-8)(emphasis added):

> A plan and initial work to clone the full length B.t. gene that contained the complete B.t. protein coding sequence but only minimal flanking sequences is outlined at Bowdish notebook pages 3064949 (Exhibit 17) and 3064950 (Exhibit 18), both dated July 17, 1985, and 3014951 (Exhibit 19) dated July 19, 1985. This plan contemplated isolating the full length B.t. gene after digestion with AhaIII (used interchangeably with DraI which cuts at the same site) and adding linkers to this fragment. The reference to "truncated" B.t. on those pages meant only that the gene to be cloned was less than the greater than full length gene isolated by the Monsanto employee from the B.t.k. HD-1 strain and placed, for example, in pMAP4, pMAP17, and pMON294 (also known as T419) but would still encode the full length B.t. protein.
>
> During the rest of July 1985, Katherine Bowdish performed RNA analysis of tobacco plants containing a B.t. gene. Work continued on construction of a plant transformation vector containing the full length B.t. gene the first week of August 1985. Bowdish notebook page 3064967 (Exhibit 20).
>
> This work involved the digestion of pMON294 with AhaIII (or DraI), addition of linkers containing BglII sites, and ligation of this fragment containing a full length B.t. gene into BglII-digested pMON316.
>
> On September 11, 1985, a full-length B.t. gene clone in plant transformation vector pMON316 was identified and named pMAP1204. See Bowdish notebook page 3064996 of that date. (Exhibit 21).

Thereafter, on page 10 of the Fischhoff-Rogers Declaration, the following statement appears (FR 10):

> Triparental Agrobacterium matings involving pMAP1204 containing the full length B.t. gene were begun by Katherine Bowdish on January 29, 1986. See Bowdish notebook page 3208872 (Exhibit 29).

Indeed, No. 3208872, entitled "Matings," signed by Kathy Bowdish on January 29, 1986, signed as read and understood by Kathy H* on January 31, 1986, reads in pertinent part (F-RX 29)(* = unclear):

> Matings ->
> Set up cultures, pour LB plates fresh
>
> pMON1204, 6, 8 -> 5ml + Sp** (1/100 dil) 50µl/5ml] 37°C
> 2013 (both Nancy's and ***y's) 5ml + Kan (1/100)]
> ASE-1 => 5ml + cm, Kan (1/20)] 28°C
> A208-1 => 5ml +cm (1/20dil) 0.25ml/5ml]
>
> Cultures grown half of day to ~ 0.2 OD or a little higher
> Mix 0.2ml each type for mating in eppingdorf, -> re**** in 0.2ml LB
> Sp*t onto B ->
>
> Mixes | On/28°C
> 1204 + 2013(NH) + ASE-1 | Nextday, Scoop up cells w/loop
> 1206 | | | from plate surface & *****
> 1208 | | | out aliquots onto Selective
> 1204 + 2013(NH) + A208-1 | plates
> 1206 | | |
>
> 1208 | | | ASE's get 4 drugs:
> 1204 + 2013(HK) + ASE-1 | Sp, St, Kan, Cm
> 1206 | | |
> 1208 | | | A208's get 3 drugs:
> 1204 + 2013(HK) + A208-1 | Sp, St, Cm
> 1206 | | |
> 1208 | | |

> 1-30-86 Mating on LB plates -> had big spot of cell growth on each plate. Scooped this up & put into 2ml LB, vortexed & plated 100µl each onto selective plates. **** 50µl, 100µl, 200µl of one to see difference (1204X2013NHXA208)
>
> See results pg 3208875[.]

On page 7 of the Bowdish Declaration, the following statement appears (FR 22):

> During the period of September 1985 to January 1986, work was ongoing [sic, to] confirm that the full length B.t. gene used in pMAP1204 was functional by examining the expression of this gene in E. coli.
>
> Triparental Agrobacterium matings involving pMAP1204 containing the full length B.t. gene were begun by me on January 29, 1986. See Bowdish notebook page 3208872 (Exhibit 20).
>
> On February 7, 1986, B.t. mRNA was detected by a northern analysis of polyA+ RNA from tomato callus transformed with pMAP1202 containing truncated gene (Bowdish notebook page 3208879 (Exhibit 21)). A whole plant RNA analysis was begun on plants 337 and 344 (another plant transformed with pMAP1202) for the truncated B.t. gene transcript of February 12, 1986. Bowdish notebook page 3208886 (Exhibit 22). Results of the analysis appeared on February 21, 1986, indicating that an RNA encoding a truncated B.t. gene was present in these plants. Bowdish notebook page 3208898 (Exhibit 23).

Bowdish Exhibits 20, 21, 22, and 23 are identical to Fischhoff-Rogers Exhibits 29, 30, 31, and 32, respectively. On pages 9-11 of the Fischhoff-Rogers Declaration (FR 9-11; FR 22), we find statements substantially identical to those on page 7 of the Bowdish Declaration (FR 9-11). Interestingly, at the bottom of

Bowdish Exhibit 21/Fischhoff-Rogers Exhibit 30, i.e., Bowdish's notebook No. 3208879 entitled "Northern," we find the following entry, "*Results -> Tomato polyA+ shows full length Bt!" This entry appears to be inconsistent with Fischhoff-Rogers/Bowdish Declarations (FR 10-11; FR 22) that "B.t. mRNA was detected by a northern analysis of polyA+ RNA from tomato callus transformed with pMAP1202 containing truncated gene" (FR 10; FR 22) and "[r]esults of the analysis appeared on February 21, 1986, indicating that an RNA encoding a truncated B.t. gene was present in these plants. Bowdish notebook page 3208898 . . ." (FR 11; FR 22).

The handwritten entries of Fischhoff-Rogers Exhibit 32/ Bowdish Exhibit 23, notebook No. 3208898, entitled "Northern - leaves," signed February 21, 1986, and signed as read and understood by Kathy H*, are reproduced in pertinent part below (* = unclear):

> 2-22-86
> Results: Looks good!
> Tomato #337 with 20µg PolyA+ shows strong Bt band. It also has two lower MW Bands present.
>
> Scy+/-Kan -> no presence of Bt message on film
>
> Tomato #344 -> looks very faint for Bt. See longer exposure

Tomato #337 -> 5µg polyA+ -> can see ~ 4X dou**
from 20µg lane of Bt message

. . . .

3-3-86 -> 9 day exposure Results =>
Tomato plant #337 shows very intense bands when probing with Bt probe fragment. Plant #344 ***** Bt message present though less. . . . .

Thereafter, Fischhoff-Rogers declares (FR 11):

> There was confirmation of a successful mating into Agrobacterium of pMAP1204 containing a full length B.t. gene by Southern analysis on February 25, 1986. Bowdish notebook page 3448106 (Exhibit 33, a two page exhibit).

Fischhoff-Rogers Exhibit 33 (Bowdish notebook No. 3448106), entitled "Southern Analysis," reports the following results (F-RX 33, pp. 1-2):

Results -> all look correct except lane 6 (1206 ASE C) and lane 11 (A208 1208A)
But have one correct one for each type Δ choose:
(2) 1204 ASE B
(3) 1204 A208 B
(5) 1206 ASE B
(7) 1206 A208 A
(9) 1208 ASE (pool) A?
(12) 1208 A208 B
intermed. vector bands are much stronger than junction fragments - not much homology here - also could be tandem duplication* *There seem to be tandem duplications in all matings here because the large Bam fragment shows in all cases ->

Fischhoff-Rogers declares (FR 11):

> There were positive results from insect toxicity assays evidencing insect resistance in plants 337

> and 344 on February 28, 1986. The tests were run by Pamela G. Marrone, at that time the Group Leader, Insect Biology of Monsanto. Fischhoff indicated to Pamela Marrone what plants were to be insect tested. Marrone notebook page 2586981 (Exhibit 34)[(F-RX 34)].

Primarily as it relates to testing tomato plants, Marrone notebook No. 2586981, entitled "Test#1 Tobacco + Tomato leaves gen. eng. with B.t. gene," signed February 28, 1986, by Pamela Marrone, and illegibly signed as read and understood by another on or about May 7, 1986, reads:

> Newly hatched Manduca sexta (tobacco hornworm[)] were added to tobacco leaves and tomato plants. Tomato plants (10/plant, covered with plastic milk containers capped with black mesh netting. 25°C 14/10
>
> . . . .
>
> TOMATO
> 306 TMV 3/10 4/10 7/10 2/10 = 16/40 surv = 48% CM
> T30 6/10 9/10 10/10 6/10 = 31/40 = 78% Surv.
> 344/1202 0/10 1/10* 3/10* 0/10 *weight loss = 4/40 = 87% CM
> 337/1202 0/10 0/10 2/10 2/10 * " " = 4/40 = " "
>
> Conclusions = Tobacco with B.t. did not cause mortality greater than controls. Tomato plants with B.t. had a sign. effect on hornworm larvae.

We note that the above-mentioned insect toxicity assays refer to tomato plants 344/1202 and 337/1202 regenerated from tomato plant cells transformed by the pMAP1202 transformation vector comprising a truncated form of the Bt crystal protein gene which

does not appear to be capable of encoding a Bt crystal protein of about 130 kD (FR 11; FR 22). This evidence establishes that tomato plants regenerated from tomato plant cells transformed by a truncated Bt crystal protein gene had a significant effect on hornworm larvae.

Having considered all the foregoing information and statements, Fischhoff-Rogers declared (FR 11-12)(emphasis added):

> Initiation of tomato transformation with Agrobacterium containing full-length B.t. gene was begun on March 3, 1986 by Jeanne Layton (see Layton notebook page 338884 [sic, 3388884] (Exhibit 35) and two days later there was incubation of T3 and T30 cotyledons with Agrobacterium cultures containing pMAP1204. The purpose of the work recited at the top of notebook page 3388884 was "[t]o generate Bacillus thurgiensis [sic] gene variant tomato plants so that plants containing various lengths of the gene can be assayed for toxicity to the worms and analyzed biochemically."
>
> Layton notebook page 3388884 shows also a construct containing pMAP1206; it is referred to on the page as "1206." This construct was another truncated B.t. gene. There were four plant expression vectors in the pMAP1200 series that contained different segments of the B.t. gene. pMAP1202 was truncated at the KpnI site. pMAP1204 contained a full-length coding sequence and only a minimal non-coding flanking sequence. pMAP1206 was truncated at the Bc1I site and encodes a toxic B.t. protein shorter than the B.t. protein in pMAP1202. pMAP1208 was truncated at the HindIII site and includes a non-toxic B.t. protein.

Handwritten notebook No. 3388884 (Fischhoff-Rogers Exhibit 35/ Layton Exhibit 9), which is entitled "TT 3/7/86," signed March 3, 1986, by Jeanne G. Niedermeyer [Layton], and signed as read and

understood by Nancy L. Hoffmann on March 26, 1986, is reproduced in pertinent part below (F-RX 35/LX 9)(emphasis added):

> Purpose:
>
> To generate Bacillus thurgiensis [sic] gene variant tomato plants so that plants containing various lengths of the gene can be assayed for toxicity to the worms and analyzed biochemically
>
> Constructs are as follows:
> (All are on the pMON316SE cassette vector:)
>
> (Cis form)
> 3111SE x 1204 = terminated BT at Aha site in 35S cassette
> = full length + 10bp downstream
> 3111SE x 1206 = Truncated Bt at BclI site (35S)
> +/- toxicity
> 53% of gene.
>
> 3111SE x 1208 = truncated Bt at HindIII site (35S)
> 46% of gene.
> (we expect this gene product to be non-toxic)
>
> 8053SE = 35S/full length
> 1202SE = 35S/truncated
> Also to generate EPSP construct transgenic plants
> 3/5/86 Incubate T3 cots most look really healthy/~5% damaged
> T30 cots - only a few
> Start cultures in S/S/Kan/Cap
> *1202ASE) from Cathy
> *1204ASE) Bowdish
> 1206ASE) plates
> 1208ASE)

Regarding Fischhoff-Rogers Exhibit 35/Layton Exhibit 9, the Jeanne G. Layton Declaration (FR 26-27) looks to be a restatement of the Fischhoff-Rogers Declaration (FR 11-12).

According to Fischhoff-Rogers (FR 12) and Layton (FR 27):

> Tomato leaf pieces were innoculated with pMAP1204 ASE containing the full-length B.t. gene on March 12, 1986; tomato cotyledons were similarly inoculated the next day. See Layton notebook page 3388892 ([F-R] Exhibit 36[/LX 10]) and Layton culture book for tissue 380 ([F-R] Exhibit 37[/LX 11]).

Fischhoff-Rogers Exhibit 36/Layton Exhibit 10, entitled

"Tomato Transformation Leaf 3/12/86 and Cotyledon TT3/13/86," signed by Jeanne G. Niedermeyer [(Layton)] on March 6, 1986, and signed as read and understood by Nancy L. Hoffmann, on March 26, 1986, reads (* = unclear):

3/6/86 germinate T3 and T8 seeds -

Purpose: Generate Bacillus thuringiensis callus, plants.

3/10/86 Incubate surface sterilized T30 leaf tissue from ~ 6wk old plants the Bt constructs mentioned p. _____ to generate lots of callus tissue.

3/11 incubate T3/T8 Cots for transformation

Start Agro Cultures
(1202 ASE)
(1204 ASE)
(1206 ASE)
(1208 ASE)
(8053 SE)-from my glycerol
(8053 SE)->from glycerol Set B since pink bacteria showed up on TT 3/7/86 8053 set of plates.

3/12/86 Inoculate T30 leaf pieces with 1202 ASE, 1204 ASE, and 8053 SE(B)[All leaves look fairly healthy. no water damage]

restart cultures in 3/8/86 LB s/s/KM/***
streak 8053 SE onto plate

3/13/86 Inoculate cotyledons with
(1202 ASE
(1204 ASE and 8053 SE

3/14/86 Check 8053 SE -plate and find some pink colonies coming up. Note that there may have been a problem with original glycerol since the A glycerol also showed pink contamination. transfer leaf sections to selection plates.

Fischhoff-Rogers Exhibit 37/Layton Exhibit 11 is an undated, unsigned tabulation including, as we read the table, the following data:

Tissue #	Genotype	Construct Inserted	Transf. Date	Root on Km	Date in soil
380	T8	1204 ASE	TT 3/13	Yes	5/19

NOP Assay	Leaf Assay	Progeny Segregation	Phenotype
5/23 pos Repeated 5/29	6/9 Pos 6/20	Pos 6/29	discarded 12/4 but saved 3 prog.

Notebook No. 3449622, entitled "Insect Toxicity Assays with pMAP1202 Transformed Tobacco + Tomato," signed by David Fischhoff on March 14, 1986, and signed as read and understood by D M Shah on March 27, 1986, incorporates Marrone's notebook No. 2586981 (F-RX 34) and reports with respect to the tomato entries therein (F-RX 38):

Insect toxicity Assays performed by Pam Marrone on leaves +/or cuttings of pMAP 1202 transformed tobacco + tomato - pMAP1202 = 35S - B.t.(Kpn O)-NOS

. . . . .

Tomato
#344 + 337 = pMAP1202
#306 = 35S-TMV coat
(no B.t.)
T-30 = nontransformed

All tomatoes are T-30 variety.

Notebook No. 3449623, entitled "Insect Toxicity Assays (p. 2)," signed March 14, 1986, by David Fischhoff, and signed as read and understood by D M Shah on March 27, 1986 (F-RX 39), reports (emphasis added):

Data on previous page indicates:

1) Both pMAP1202 tomato plants, #337 + #344 have significant toxicity to Manduca sexta neonates. Significantly higher than T30 or # 306 (TMV coat)

2) No significant toxicity seen in any tobacco plants. This is consistent w/ inability to detect, so far, any B.t.-specific RNA from these tobacco plants.

3) Northerns indicate # 337 has more B.t.-specific mRNA than # 344. Both seem equally toxic in this test.

4) In this test, background (control) mortalities were relatively high.

Notebook No. 3451066, entitled "Nop Assay," signed May 23, 1986, by Jeanne G. Niedermeyer (Layton), and signed as read and

understood by Nancy L. Hoffmann, on May 27, 1986, reports positive results for plant # 380 (F-RX 40/LX 12). Notebook No. 3451079, entitled "Leaf Assays on 1^{o} Transformants," signed June 9, 1986, by Jeanne G. Niedermeyer (Layton), and signed as read and understood by Nancy L. Hoffmann on July 26, 198?, reports positive results for plant # 380 on 6/20/86 (F-RX 41/ LX 13).

Notebook No. 3465067, entitled "New Transgenic B.t. Tomato Plants," signed by David Fischhoff on June 24, 1986, and signed as read and understood by Cathy Hironara on August 4, 1986 (F-RX 42), reports:

> New transgenic tomato plants with B.t. constructs, now large enough for toxicity tests.
>
> | Plant | Construct | Tomato Genotype | Nopaline | Kan^{R} leaf assay |
> |---|---|---|---|---|
> | 378 | 1202 | T3 | - | + |
> | 380 | 1204 | T8 | + | + |
> | 381 | 1202 | T8 | - | - at 15 days |
> | 379 | pMON200 | T8 | - | - at 15 days |
>
> All of these given to Pam Marrone for Manduca sexta toxicity tests as whole plants - 6/24/86
>
> | | | | | |
> |---|---|---|---|---|
> | 371 | 1202 | T30 | Not tested | - |
>
> 371 is an old transformant that was negative for Kan^{R} but hadn't been tested for toxicity or for nopaline.
>
> Tested now as leaf cuttings for toxicity + J. Niedermeyer will do nopaline.

Thereafter, Fischhoff presents its evidence that tomato plant #380, which shows positive under Nop and Kan^R leaf assays, was transformed by a 1204 construct comprising a full length B.t. crystal protein gene encompassed by pMON294/DraI (equivalent to T419/DraI, pMON294/AhaIII, and T419/AhaIII) under control of the CaMV35S promoter in pMON316, so that the full length B.t. crystal protein gene is expressed in tomato plant #380 in amounts insecticidal to Manduca sexta, Lepidopteran insects. The handwritten entries in notebook No. 3158615 (F-RX 43/ Marrone Exhibit 3(MX 3)), entitled "Test of Gen. Eng. Tomatoes," signed by Pamela G. Marrone on June 27, 1986, and signed as read and understood by S. R. Sims (sic?) on January 9 1987, read (* = unclear):

380		1 alive, 9 dead
344	28	2 alive, 8 dead
379		2 alive 2 dead, remainder missing
381		1 6 dead, remainder missing
378		0 8 dead, 2 missing
371		all dead)
		all dead) in dishes (10/10)
		all dead)
344	64	5 alive, 5 dead
344	9	1 alive, 8 dead, 1 missing
344	11	8 alive, 2 missing
344	51	9 alive, 1 dead

All tests, except 371, done on whole plants. Plants covered with plastic milk containers******** added, Screen ***** placed on top. Test read after * days.

The results reported in Marrone's notebook No. 3158615 (F-RX43/ MX 3) are reentered in notebook No. 3465071 (F-RX 44), entitled "Results of Insect Assays - M. sexta vs. New Tomatoes + 344 F1's," signed June 29, 1986, by David Fischhoff, and signed as read and understood by Cathy Huonara (Sic?) on August 4, 1986, as follows (F-RX 44)(* = unclear):

> M. sexta feeding assays by P. Marrone done as whole plants in jars for all but 371 - too big to fit.
> See p. 067 for plant data.
> Repeated also 4 344 F1's. This is a retest of some on pp. 065-066.
> That test was on 6/2-6/5/86
> This test is 6/25-6/29/86 = 3 wks later
>
> These were planted 5/9/86 + have been in growth chamber
>
> Greenhouse plants

	Alive	Dead	Missing	Vector	Nop	Kan
378	0	8	2	1202	-	+
379	2	2	6	200	-	-
380	1	9	0	1204	+	+
381	1	6	3	1202	-	-
371	0	10)		1202	-	-
	0	10)	3 dishes			
	0	10)				
				Previous % CM		
344-28	2	8	(80%)	100%		
344-64	5	5	(50%)	100%		
344-9	1	8	1 (90%)	100%		
344-11	8	2	(20%)	0%		
******	*	*	(10%)	10%		

> Notebook No. 3465076 (F-RX 46), entitled "Manduca Sexta Assay - Repeat of Assay of p. 3465071," signed by David Fischhoff

on July 8, 1986, and signed as read and understood by Cathy Huonara (sic?) on August 4, 1986, reads (F-RX 46):

Repeated Manduca sexta assay on new transformed plants - p. 3465071
Assay done on cut leaves in Petri dishes. see p. 073

Plant	Larvae alive	Larvae dead		
379	2	8	200	Nop-Kan^S
	0	10		
	2	8		
	0	10		
	4	36		
378	8	2	1202	Nop-Kan^R
	0	10	(old leaves)	
	8	2		
	2	8		
	18	22		
380	1	9	1204	Nop+Kan^R
	9	1		
	0	10		
	1	9		
	11	29		
381	0	10	1202	Nop-Kan^S
	1	9		
	5	5		
	8	2		
	14	26		
371	0	10	1202	Nop-Kan^S
	2	8		
	0	10		
	0	10		
	2	38		
364	0	10	554	Nop+Kan^R
	0	10		
	0	10		
	0	10		
	0	40		

Notebook No. 3465082 (F-RX 47), entitled "Manduca Retest (p. 2)," signed by David Fischhoff on July 16, 1986, and signed as read and understood by Cathy Huonara (sic?) on August 4, 1986, reads (F-RX 4&)(emphasis added):

> 378 = 1202 Nop-Kan^R 364 = 554
> 380 = 1204 Nop+Kan^R 371 = 1202 nop-Kan^S
> 381 = 1202 Nop-Kan^S
>
> Results of this assay indicate that on younger 1^o transformants (378, 380, 381),
> after washing leaves only 378 + 380 remain toxic
> 381 now down to 35%
> If 381 is really nontransformed (it is Nop-Kan^S) then it represents a negative control.
> Thus, <u>both 378 (1202) + 380 (1204) are showing evidence of B.t. toxicity</u>.[3]
> Due to insecticide problems we need to confirm this again.
>
> However, w/ older 1^o transformant, 371 + 364, washing leaves did not reduce toxicity.
>
> 364 has no B.t. gene + is still killing 100%.
> These plants have been in greenhouse for >6 months + perhaps have a high residue of insecticide.
>
> Thus the results for 371 are uncertain.
> It is Nop-Kan^S so perhaps it's a negative, also.
>
> We will need to assay progeny of 371 to test toxicity.

---

[3] The evidence establishes that tomato plants regenerated from tomato plant cells transformed by pMAP1202 (a KpnI truncated <u>Bt</u> crystal protein gene) and pMAP1204 (a DraI/AhaIII truncated <u>Bt</u> crystal gene containing a full-length coding sequence and minimal non-coding flanking sequence) show evidence of <u>Bt</u> toxicity toward Lepidopteran insects.

In notebook No. 3480151 (F-RX 48), entitled "BT Variant Constructs," signed by Jeanne G. Niedermeyer (Layton) on 8/19, and signed as read and understood by Nancy L. Hoffmann on September 26, 1986, we find the following conclusions (F-RX 48)(emphasis added):

> 8/20/86
> Plant 380 X) for BT project
> 381 X)
>
> 380 is a pMON1204 ASE which is expressing the BT toxin as judged by the feeding assay.
> 381 X produced questionable results in feeding assay : progeny will be assayed.

Finally, in notebook No. 3158646 (F-RX 49/MX 4), entitled "Test of Tomato plants (Gen. Eng.) vs. *****," (* = unclear), signed October 6, 1986, by Pamela G. Marrone, and signed as read and understood by S. R. Sims (sic?) on January 9, 1987, we find the following results reported (F-RX 49/MX 4)(* = unclear):

> 10 larvae per plant added to tomato seedlings, then covered with plastic milk containers. Small plants had only 5 larvae added.

	#alive/#added		#alive/#added
337-87 X 15	3/10 = 66.7%	337-87 X 3	0/10 = 100%
337-87 X 20	0/10 100	337-87 X 4	2/10 = 77
337-87 X 9	2/10 77.8	337-87 X 28	0/10 = 100
337-87 X 12	0/10 100	337-87 X 11	1/10 = 88*
337-87 X 19	1/10 88.9		
337-87 X 24	1/10 "		
337-87 X 7	0/10 100		
337-87 X 14	0/10 100		

337-87 X 1		1/10	88.9			
337-87 X 2		1/10	88.9			
380 X	3	5/10	44.4	380 X	2/5 =	55.6%
380 X		4/10	55.6	380 X	3/10	66.7
380 X		4/10	"	380 X	5/10	44.4
380 X	6	5/10	44.4			
380 X		3/5	(Small plant)			
380 X	1	5/10	44.4			
380 X	4	0/10	100			
380 X		3/10	66.7			
380 X	2	7/10	22.2			
380 X	11	6/10	33.3			
380 X	12	4/10	55.6			
380 X	9	4/10	"			
380 X	5	5/10	44.4			
380 X	6	4/10	55.6			
380 X	7	4/10	55.6			
380 X		4/10	55.6			
380 X		2/5	(Small plant) = 55.6			
380 X	8	4/10	55.6			
380 X		5/5	(Small plant) = 0			
380 X		4/10	55.6			

In notebook No. 3547849 (F-RX 51), entitled "Repeat Manduca Assays (p. 3)," signed October 28, 1986, by David Fischhoff, and signed as read and understood by Dannette C. Ward on November 6, 1986, there are found the following conclusions (F-RX 51) (emphasis added):

> Conclusions for 381 X + 38 X [(sic, 380 X)]
>
> For 381 X there is no significant toxicity.
> Test of 10/6/86 had higher overall mortality than 10/28/86 but best plants from each test did not reproduce well in other test. Given that this is Nop- Kan^S, conclude that it's probably nontransformed or nonexpresser of all genes.

For 380 X there is low but consistent toxicity.
Level of toxicity is lower than for 337 F1's + F2's but is apparent.
It may be that higher tox F1's are homozygotes.
There is a rough correlation of nopaline negatives (2 plants) w/ nontoxicity
Compared to 381 X there is good repeatability of 2 tests.
Control mortality was low in both assays making tests more believable.

This indicates that 1204 (pMON9712) can be expressed at low but detectably toxic level.

The direct testimony of David A. Fischhoff and Stephen G. Rogers (Fischhoff-Rogers)(e.g. FR 1-15), and their associates Katherine S. Bowdish (Bowdish)(e.g. FR 16-23), Jeanne G. (Niedermeyer) Layton (Layton)(e.g. FR 24-28), and Pamela Gail Marrone (Marrone)(e.g. FR 29-30), all supporting exhibits (especially the handwritten laboratory notebooks (F-RX 2-51; BX 2-23; LX 2-13; and MX 2-4)), cross-examination relative thereto, Fischhoff '86 (filed November 20, 1986), and Fischhoff '91 (filed December 23, 1991), as a whole, support the following findings:

(1) On April 4, 1985, and again on June 17, 1985, B.t. DNA-containing T419 and B.t. DNA-containing pMON294 were identified as the same vector (F-RX 12/ BX 9; F-RX 13/BX 10).

(2) On June 17, 1985, Bowdish reported that the B.t. DNA fragment cut from T419/pMON294 using AhaIII/DraI "has to be at least 3470bp long" (F-RX 13/BX 10).

(3) Fischhoff-Rogers declared that Bowdish's laboratory notebook dated July 17, 1985 (F-RX 17/BX 15) describes a plan "to clone the full length B.t. gene that contained the complete B.t. protein coding sequence but only minimal flanking sequences" (FR 7) and Bowdish's laboratory notebooks dated July 17, 1985, July 19, 1985, and August 5, 1985 (F-RX 18-20/BX 16-18) report "isolating the full length B.t. gene after digestion with AhaIII (used interchangeably with DraI which cuts at the same site) and adding linkers to this fragment" (FR 7).

(4) On September 11, 1985, Bowdish described cutting B.t. DNA in T419/pMON294 using AhaIII/DraI, adding BglII linkers thereto, cutting pMON316 with BglII, and ligating AhaIII/DraI cut B.t. DNA with added BglII linkers to BglII cut pMON316 at the BglII sites (F-RX 17-21/BX 15-19).

(5) On September 11, 1985, Bowdish identified pMAP1204 as a clone of a product formed by cutting B.t. DNA in T419/pMON294 with AhaIII/DraI, adding BglII linkers thereto, and ligating AhaIII/DraI cut B.t. DNA with added BglII linkers to BglII cut pMON316 (F-RX 21/BX 19).

(6) Bowdish's laboratory notebooks support Fischhoff's declaration that (FR 8):

> The reference to "truncated" B.t. on those pages meant only that the gene to be cloned was less than the greater than full length gene isolated by the Monsanto employee from the B.t.k. HD-1 strain and placed, for example, in . . . pMON294 (also known as T419) but would still encode the full length B.t. protein.
>
> . . . . .
>
> This work involved the digestion of pMON294 with AhaIII (or DraI), addition of linkers containing BglII sites, and ligation of this fragment containing a full length B.t. gene into BglII-digested pMON316.
>
> On September 11, 1985, a full-length B.t. gene clone in plant transformation vector pMON316 was identified and named pMAP1204. See Bowdish notebook page 3064996 of that date. (Exhibit 21).

(7) On March 3, 1986, Layton identified the 3111SE X 1204 construct as "= terminated Bt at Aha site in 35S = full length + ~10bp downstream" or full length B.t. gene under control of the CaMV35S promoter (F-RX35/LX 9).

(8) Fischhoff-Rogers declared (FR 11-12):

> Initiation of tomato transformation with Agrobacterium containing full-length B.t. gene was begun on March 3, 1986 by Jeanne Layton (see Layton notebook page 338884 [sic, 3388884] (Exhibit 35) and two days later there was incubation of T3 and T30 cotyledons with Agrobacterium cultures containing pMAP1204. The purpose of the work recited at the top of notebook page 3388884 was "[t]o generate Bacillus thurgiensis [sic] gene

variant tomato plants so that plants containing various lengths of the gene can be assayed for toxicity to the worms and analyzed biochemically."

Layton notebook page 3388884 shows also a construct containing pMAP1206; it is referred to on the page as "1206." This construct was another truncated B.t. gene. There were four plant expression vectors in the pMAP1200 series that contained different segments of the B.t. gene. pMAP1202 was truncated at the KpnI site. pMAP1204 contained a full-length coding sequence and only a minimal non-coding flanking sequence. pMAP1206 was truncated at the BclI site and encodes a toxic B.t. protein shorter than the B.t. protein in pMAP1202. pMAP1208 was truncated at the HindIII site and includes a non-toxic B.t. protein.

(9) Laboratory notebook No. 3388884, which is entitled "TT 3/7/86," signed March 3, 1986, by Jeanne G. Niedermeyer [Layton], and signed as read and understood by Nancy L. Hoffmann on March 26, 1986 (F-RX 35/LX 9), and Laboratory notebook No. 3388892, which is entitled "Tomato Transformation 3/12/86 Leaf and TT 3/13/86 Cotyledon," signed March 6, 1986, by Jeanne G. Niedermeyer [Layton], and signed as read and understood by Nancy L. Hoffmann on March 26, 1986 (F-RX 36/LX 10), support the Fischhoff-Rogers declaration (FR 11-12).

(10) Fischhoff-Rogers (FR 12) and Layton (FR 27) declared that T30 tomato leaf pieces and T3/T8 cotyledons were innoculated with Agrobacterium cultures containing the pMAP1204 construct containing the full-length B.t. gene on March 12 and 13, 1986.

(11) Laboratory notebook No. 3388892 which is entitled "Tomato Transformation 3/12/86 Leaf and TT 3/13/86 Cotyledon," signed March 6, 1986, by Jeanne G. Niedermeyer [Layton], and signed as read and understood by Nancy L. Hoffmann on March 26, 1986 (F-RX 36/LX 10), supports the Fischhoff-Rogers and Layton declarations (FR 12/FR 27).

(12) Fischhoff-Rogers (FR 12/FR 27) declared that the results of the March 12/13, 1986, innoculation of T30 tomato leaf pieces and T3/T8 cotyledons with Agrobacterium cultures containing pMAP1204 were reported in Layton culture book for tissue 380 (F-RX 37/LX 11).

(13) Layton culture book (F-RX 37/LX 11) defines Tissue #380 as tomato tissue having been generated from T8 tomato plants inoculated with a 1204 construct on March 13, 1986, rooted on Km, and transferred to soil on May 19, 1986, which showed positive under Nop and Leaf Assays indicative of tomato plant cell transformation by the B.t. crystal protein gene under control of the CaMV35S promoter in the 1204 construct.

(14) Fischhoff declared that (FR 13):

> [a] tomato plant (number 380) transformed with pMAP1204 was placed in soil on May 19, 1986. Four days later there was a summary of positive NOP (nopaline) assays for plant 380 containing the full-length B.t. gene. . . . .

> Kanamycin resistance assays on plant 380 were begun on June 9, 1986 with a positive leaf assay result reported for that plant on June 20, 1986. . . . .

(15) Layton described positive results for Nop Assay of 1^0 Transformants of Tomato Plant #380 on May 23, 1986 (F-RX40/ LX 12).

(16) Layton described positive results for Leaf Assays on 1^0 Transformants of Tomato Plant #380 on June 9, 1986 (F-RX41/ LX 13).

(17) Fischhoff declared that he "listed plants including one transformed with pMAP1204 (plant 380) that were ready for toxicity testing" (FR 13) in notebook No. 3465067 on June 24, 1986 (F-RX 42), and that "three days later there were results of insect toxicity studies on plant 380 evidencing insecticidal activity" (FR 13). He declared that his observations on those toxicity studies were recorded in notebook No. 3465071 on June 29, 1986 (FR 13-14).

(18) In notebook No. 3465067, dated June 24, 1986, entitled "New Transgenic B.t. Tomato Plants," co-signed by another on August 4, 1986, Fischhoff listed the following "[n]ew transgenic tomato plants with B.t. constructs, now large enough for toxicity tests" (F-RX 42):

Plant	Construct	Tomato Genotype	Nopaline	Kan^R leaf assay
380	1204	T8	+	+

Fischhoff also reported that transgenic tomato plant #380 was "given to Pam Marrone for Manduca sexta toxicity tests as whole plants - 6/24/86" (F-RX 42).

(19) Marrone declared that tomato plant #380 showed positive results from insect toxicity assays reported in notebook No. 3158615 dated June 27, 1986 (FR 29-30).

(20) Marrone reported "1 alive, 9 dead" for 380 in a "Test of Gen. Eng. Tomatoes" dated June 27, 1986 (F-RX 43/MX 3).

(21) Fischhoff entered the following comments and "Results of Insect Assays - M. sexta vs. New Tomatoes + 344 F1's" (F-RX 44), including the results of "M. sexta feeding assays by P. Marrone done as whole plants" (F-RX 44) reported in notebook No. 3158615 (F-RX 43/MX 3), in Fischhoff notebook No. 3465071 dated June 29, 1986 (F-RX 44):

Greenhouse plants	Alive	Dead	Missing	Vector	Nop	Kan
380	1	9	0	1204	+	+

(22) Fischhoff declared that a retest of the insect toxicity studies for tomato plants #380 said to contain a full-length B.t. crystal protein gene began on July 8, 1986 (FR 14). Fischhoff declared that "results from that retest conducted by Pamela

Marrone were reported on July 16, 1986 indicating that the plant was still insecticidal," citing Fischhoff notebook Nos. 3465076 (F-RX 46) and 3465082 (F-RX 47)(FR 14).

(23) Fischhoff notebook No. 3465076, entitled "Manduca Sexta Assay - Repeat of Assay of p. 3465071 [(F-RX 44)]," dated July 8, 1986, reports that a Manduca sexta assay on new transferred plants was done on cut leaves in Petri dishes with the following results (F-RX 46):

Plant	Larvae alive	Larvae Dead	Vector	Nop	Kan
380	1	9	1204	Nop+	Kan^R
	9	1			
	0	10			
	1	9			[.]

(24) Fischhoff notebook No. 3465082, entitled "Manduca Retest (p. 2)," dated July 16, 1986, on retesting tomato plant "380 = 1204 Nop+ Kan^R" (F-RX 47), reports that only the washed leaves of tomato plants 378, 380 and 381 remained toxic to Manduca sexta (F-RX 47). However, Nop- and Kan^R assays for tomato plant 381 indicate that it was nontransformed and thus suitable as a control (F-RX 47). Fischhoff concluded that tomato plant 378 had been transformed using the pMAP1202 vector containing a truncated B.t. gene and tomato plant 380 had been

transformed using the pMAP1204 vector containing full-length B.t. gene (F-RX 47).

(25) Layton again identified "Plant 380" as "a pMON 1204 ASE which is expressing the BT toxin as judged by the feeding assay" in a laboratory notebook entry dated August 20, 1986, and co-signed by an associate on September 26, 1986 (F-RX 48).

(26) Fischhoff-Rogers declared that "there were reported positive results from insect toxicity assays evidencing insect resistance of progeny of plant 380" on October 6, 1986 (FR 14).

(27) Marrone declared that "there were reported positive results from insect toxicity assays evidencing insect resistance of progeny of plant 380" on October 6, 1986 (FR 30).

(28) Marrone reported positive results from repeated insect toxicity assays of a large number of #380 tomato plants in her laboratory notebook No. 3158646 dated October 6, 1986 (F-RX 49/ MX 4).

(29) Fischhoff entered a conclusion and summary of data for progeny of #380 tomato plants in Fischhoff notebook No. 3547849 dated October 28, 1986 (F-RX 51).

(30) The #380 tomato plants regenerated from tomato plant cells transformed using pMAP1204 containing an AhaIII/DraI truncated B.t. crystal protein gene at least 3470bp long under

control of a CaMV35S promoter showed a consistent level of toxicity toward Manduca sexta comparatively higher than the level of toxicity of nontransformed control tomato plants toward Manduca sexta and comparatively lower than the level of toxicity of tomato plants transformed using pMAP1202 containing a KpnI truncated B.t. crystal protein gene less than 3470bp long toward Manduca sexta no later than October 28, 1986 (F-RX 51).

(31) The #380 tomato plants regenerated from tomato plant cells transformed using a Nop+ Kan^R pMAP1204 comprising an AhaIII/DraI truncated Bt crystal protein gene at least 3470bp long, produced Bt crystal protein in amounts insecticidal to Lepidopteran insects no later than October 28, 1986 (F-RX 13/BX 10; F-RX 37/LX 11).

(32) Tomato plant #337 regenerated from tomato plant cells transformed using Nop- Kan^R pMAP1202 comprising a KpnI truncated Bt crystal protein gene substantially less than 3470bp long (KpnI digests indicate that the KpnI truncated Bt crystal protein gene is ~2175bp long (F-RX 21/BX 19)), produced Bt crystal protein in amounts insecticidal to Lepidopteran insects no later than October 28, 1986 (F-RX 39).

(33) The #380 tomato plants regenerated from tomato plant cells transformed using pMAP1204, showed a level toxicity to

Lepidopteran insects somewhat lower than the level of toxicity to Lepidopteran insects exhibited by #337 tomato plants regenerated from tomato plant cells transformed using pMAP1202 no later than October 28, 1986 (F-RX 51).

(34) On November 20, 1986, Fischhoff filed Fischhoff '86 which describes a pMON9712 construct as "comprising the full-length B.t. construct in which the structural coding sequence for the B.t. toxin was truncated at the DraI site at position 3479," a site "10 nucleotides beyond the translational terminator codon for the coding sequence for the full-length B.t. toxin" (Fischhoff '86, p. 15-16, bridging para.):

> [T]his construct contains the full-length coding sequence but very little 3' flanking sequence from the B.t. subspecies kurstaki gene. This construct is designated pMON9712.

(35) A preponderance of the evidence indicates that the pMAP1204 construct Fischhoff used to transform tomato plant cells from which it regenerated #380 tomato plants contained a B.t. crystal protein gene of about 3470bp (~3.5kb) long under control of a CaMV35S promoter which directs expression of said gene in said regenerated tomato plants in amounts insecticidal to Lepidopteran insects no later than October 28, 1986;

(36) The preponderance of the evidence establishes that Fischhoff recognized and appreciated that #380 tomato plants control Lepidopteran insects (Manduca sexta) no later than October 28, 1986.

(37) Nevertheless, the evidence of record does not establish that the #380 tomato plants had been regenerated from "a tomato plant cell transformed to comprise a full length Bacillus thuringiensis crystal protein gene capable of encoding Bacillus thuringiensis crystal protein of about 130 kD" as per Count 1.

Adang's evidence shows that Adang regenerated L-7 tomato plants which were insecticidal to Lepidopteran insects from tomato plant cells transformed to comprise a Bt toxin coding sequence approximately 3.8 kb long. Adang's evidence also shows that its L-7 tomato plants expressed a Bt crystal protein having a molecular weight of about 130 kD.

Fischhoff's evidence shows that Fischhoff regenerated #380 tomato plants which were insecticidal to Lepidopteran insects from tomato plant cells transformed to comprise a Bt toxin coding sequence less than 3.5 kb long. Fischhoff's evidence further shows that its #380 tomato plants produced Bt crystal protein of undetermined molecular weight.

Although Adang and Fischhoff both refer to the Bt crystal protein coding sequence each used to transform the tomato plant cells each used to regenerate insecticidal tomato plants as a full length Bt crystal gene, only Adang defines the full length Bt crystal protein gene as one which is capable of encoding a Bt crystal protein of about 130 kD and shows that its full length Bt crystal protein gene was capable of encoding a Bt crystal protein of about 130 kD. Fischhoff defines the full length Bt crystal protein gene it used to transform the tomato plant cells from which it regenerated #380 tomato plants which were insecticidal to Lepidopteran insects as a DraI truncate which includes the coding sequence for a full length Bt toxin which is insecticidal to Lepidopteran insects. However, the full length Bt crystal protein gene is not defined in terms of the molecular weight of the Bt crystal protein toxin it is capable of encoding. More importantly, Fischhoff has failed to establish that the Bt crystal protein gene it used to transform tomato plant cells from which it regenerated #380 tomato plants which were insecticidal to Lepidopteran insects was capable of encoding a Bt crystal protein of about 130 kD. The evidence of record as a whole establishes that both truncated and full length Bt crystal protein coding sequences are capable of encoding Bt crystal

proteins which are insecticidal to Lepidopteran insects yet have molecular weights substantially less than 130 kD. The evidence also shows that Bt crystal proteins produced by tomato plants regenerated from tomato plant cells transformed to comprise truncated Bt crystal protein genes are at least as insecticidal to Lepidopteran insects as a Bt protoxin having a molecular weight of about 130 kD of the kind produced by Adang's L-7 tomato plants. In short, Fischhoff failed to prove that the clone of the 3.5 bp Bt DNA it cut from Bt DNA at the DraI site is capable of encoding a Bt crystal protein of about 130 kD. Accordingly, Fischhoff has not established that it reduced an embodiment meeting all the limitations of Count 1 to practice on or before October 29, 1986.

Conclusions

Based on a preponderance of the evidence of record, we

CONCLUDE that regenerated tomato plants which Adang showed were insecticidal to Lepidopteran insects on October 29, 1986, were actual reductions to practice of an embodiment of Count 1;

FURTHER CONCLUDE that Adang actually reduced an embodiment meeting all the limitations of Count 1 to practice by October 29, 1986; and

FURTHER CONCLUDE that, considering all the evidence, Adang has proved it was the first to actually reduce to practice an embodiment meeting all the limitations of Count 1.

Disposition of Interference 103,324

Priority of the invention defined by Count 1 of this interference has been determined against Senior Party Fischhoff based on all the evidence of record. It is

ORDERED that, on the record before the Board of Patent Appeals and Interferences, judgment on priority of the invention of Count 1, the sole count in this interference, is awarded against Senior Party DAVID A. FISCHHOFF and STEPHEN G. ROGERS;

FURTHER ORDERED that, on the record before the Board of Patent Appeals and Interferences, judgment on priority of the invention of Count 1, the sole count in this interference, is awarded in favor of Junior Party MICHAEL J. ADANG and JOHN D. KEMP;

FURTHER ORDERED that, on the record before the Board of Patent Appeals and Interferences, Junior Party MICHAEL J. ADANG and JOHN D. KEMP is entitled to a patent containing Claims 15-17, 22, 24-27, 29-32, 34, 40 (dependent upon Claim 16, 26, 27, 29, or 30), 42 (dependent upon Claim 16, 26, 27, 29, or 30), 43 (dependent upon Claim 16, 26, 27, 29, or 30), 44 (dependent upon

Claim 16, 26, 27, 29, or 30), 46 (dependent upon Claim 16 or 26), 47-50, and 57 (corresponding to Count 1 of this interference) of Adang Application 07/713,624, filed June 10, 1991; and

FURTHER ORDERED that, on the record before the Board of Patent Appeals and Interferences, Senior Party DAVID A. FISCHHOFF and STEPHEN G. ROGERS is not entitled to a patent containing Claims 11-12, 15-16, 20-21, 24-25, 38-39 and 42-43 (corresponding to Count 1 of this interference) of Fischhoff Application 07/813,250, filed December 23, 1991.

It is also

ORDERED that if there is a settlement and it has not already been filed, attention is directed to 35 U.S.C. § 135(c) and 37 CFR § 1.661; and

FURTHER ORDERED that a copy of this decision be given an appropriate paper number and entered into the file records of Fischhoff Application 07/813,250 and Adang Application 07/713,624.

RICHARD E. SCHAFER)
Administrative Patent Judge)
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TEDDY S. GRON) BOARD OF PATENT
Administrative Patent Judge) APPEALS AND
) INTERFERENCES
)
)
JAMESON LEE)
Administrative Patent Judge)

Interference 103,324

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